



Impact of Carbamate Pesticide, Sevin on haematological parameters of Minor Carp, *Labeo boga* (Ham.)

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ABSTRACT: In the present study, an attempt has been made to evaluate the impact of carbamate pesticide, Sevin on haematological parameters in a minor carp *Labeo boga*. 96h LC₅₀ value of sevin for *Labeo boga* was found to be 1.5mg/l. Three sub-lethal concentrations viz., 20%, 40% & 60% of LC₅₀ were employed for experimental period of 60 days. The studied haematological parameters were Total Erythrocyte count (TEC), Haemoglobin (Hb), Haematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Total Leucocyte Count (TLC) and Differential Leucocyte Count (DLC). Sub-lethal concentration of sevin has been observed to cause significant dose dependent alterations in these haematological parameters thereby hampering the entire metabolic machinery of the fish.

Keywords: Sevin, *Labeo boga*, haematological parameters.

INTRODUCTION: Increased industrialization, urbanization, population growth and overall man's greed to overexploit Mother Nature has created a serious threat to all kinds of life in the form of pollution which has now become a global problem. Among all types of pollution, aquatic pollution is of greater concern as each and every organism depends on water (Rhagav *et al.*, 2019). The pollution of aquatic environment with wide array of xenobiotics has become a menace to the aquatic flora and fauna and is a problem of immediate concern. Aquatic pollutants undoubtedly have direct effect on fish health and survival. Heavy metals and xenobiotics are regarded as the serious pollutants of the aquatic environment because of their persistence and tendency to bioaccumulate in aquatic organisms particularly fishes (Hussian, 2015). Fishes are exposed to such xenobiotics present in the water by three primary ways viz., (i) skin (ii) gills and (iii) orally. The resistance of the fish to toxicity also depends on its physiological state (Pal, 2006).

Fish, a key member of many natural food webs is one of the important source of animal proteins (16-23%) and hence form a valuable commodity for human consumption (Oshode *et al.*, 2008). Fishes not only meet the food requirements of man within a country but also earn valuable foreign exchange and so fishing industries do help to provide employment to millions of people all over the world.

Stress reactions involve various physiological changes including alterations in blood composition and histopathology of various organs particularly haemopoietic and immune organs (Ololade and Ogini, 2010). Stressors can also become causative of disease outbreaks, low productivity and mortality in aquaculture. Other toxic end points include decreased growth, mobility and reproductive effects (Allen, 1994). Changes in the environmental quality can, therefore, be a major determinant of year-class strength and eventually the long-term dynamics of fish populations (Rose *et al.*, 1993). All these stresses may result in significant alteration in such important functions such as feeding, reproduction, growth, haematology etc. in fishes. Among these, haematological studies on fishes have assumed greater significance due to the increasing emphasis on pisciculture and greater awareness of the

population of natural freshwater resources. Such studies have generally been used as an effective and sensitive index to monitor physiological and pathological changes in fishes (Kayode and Shamusideen, 2010).

Haematological evaluation of fish provides valuable facts concerning the physiological response of fish to changes in the external environment. Study of haematological parameters on one hand help in establishing the health status of fish and on other is the cheapest, trusted and well known tool to monitor the ambient aquatic environment of the fish. Blood is a sensitive indicator of stress and any physiological dysfunctioning in fish's body gets reflected as alterations in its blood constituents.

Blood being the medium of intercellular and intracellular transport, comes in contact with various organs and tissues of the body and thus can pose a direct threat to physiological functions of the fish. Xenobiotics (like heavy metals/pesticides) rapidly bind to the blood proteins and thus may induce haematological changes on one hand and histopathological on the other (Tyagi and Srivastava, 2005).

Pesticides are directly applied to the agricultural fields mainly to control pests and improve the crop yield. These Pesticides finally find their way into the water bodies and cause harm to aquatic flora and fauna including fishes (Patnaik and Patra, 2006). The carbamate pesticide sevin is known to affect growth, metabolism and development of fish (Shastry *et al.*, 1988). Sublethal concentrations of sevin (Shastry *et al.*, 1988; Patnaik and Patra, 2006 and Ibrahim *et al.*, 2009) have been reported to drastically affect the haematological parameters of fish.

Presently therefore, an endeavor has been made to analyze the effect of carbamate pesticide sevin on haematological parameters viz. Total Erythrocyte Count, Haemoglobin, Haematocrit, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, Mean Corpuscular Haemoglobin Concentration, Total and Differential Leucocyte Count of fish *Labeo boga*. The effect of sublethal concentrations (20%, 40% & 60% of LC₅₀) of carbamate pesticide, sevin on the haematological parameters of fish *Labeo boga* have been evaluated for an experimental period of 60 days.

MATERIALS AND METHODS: Adult specimens of *Labeo boga* were collected with the help of cast from Nagrota stream of river Tawi from Jammu, J&K, India. The fishes were acclimatized for about 15 days. Three different concentrations viz. 20% (0.3mg/l), 40% (0.6mg/l) and 60% (0.9mg/l) of LC₅₀ of sevin along with controls were maintained during the experimental period. 0.5ml of blood was taken directly by cardiac puncture with the help of heparinised needles using EDTA as an anticoagulant. Among blood parameters TEC and TLC were counted with the help of improved Neubauer Cytometer (Shaw, 1930). Hb was determined by using Sahil's haemoglobinometer (Dethloff *et al.*, 1999) and Hct was determined by Centrifugation method (Wintrobe, 1967). MCH, MCV and MCHC were calculated by using standard formulae:

$MCV = Hct \times 10 / RBC \text{ count}$. Its unit is femtolitre (fl)

$MCH = Hb\% \times 10 / RBC \text{ counted in picogram (pg)}$

$MCHC = Hb \text{ in g/100ml} \times 100 / \text{vol. of packed RBCs in 100ml}$. It is represented as percentage (%).

Identification of Cellular Components i.e., Differential leucocyte count was done by methodology as adopted by Anderson, 2003.

Microphotography: Slides of blood smears were scanned and photographed with Sony SSC-DC378P Semi-Digital camera attached with Olympus CH20i Research Microscope

Experimental data and those of control were statistically analysed by means of analysis of variance (ANOVA). Significance was set at $P < 0.01$. All analysis was performed using SPSS software.

RESULTS and DISCUSSION:

RBCs and Related Parameters: Presently marked decline ($P < 0.01$) has been observed in TEC in sevin treated fishes compared to control (Table 1 & Fig.5) which can be attributed to i) an increase in the rate of RBC destruction and ii) abnormal/distorted shape of RBCs (Figs.) which then no longer can perform normal function. Support for the present viewpoint can be derived from the findings of Devi *et al.* (2008), Zutshi *et al.* (2009) and Palanisamy *et al.* (2011) who too observed decline in TEC as a result of erythrocyte destruction. The microscopic examination of blood smears of sevin treated fishes depicted anomalies in shape as well as nuclear malformation of RBCs (Figs. 4, 5 & 6) besides their numerical thinning. This indicates that RBC declined not only quantitatively but

qualitatively too. Distorted shape of RBCs may also result in reduced oxygen carrying capacity of RBCs and thus consequently can lead to tissue hypoxia as earlier also suggested by Das (1998), Yang and Chen (2003) and Verma (2007).

In nutshell, it has been observed that lack of synthesis of normal RBCs and their release in general circulation on one hand and presence of distorted/abnormal RBCs on the other ultimately reduces the normal functioning of RBCs in sevin treated fishes. Larson (1975), Pacheco & Santos (2002) and Tilak *et al.* (2007) in corroboration with present findings reported that stress of xenobiotics cause destruction of RBCs at quicker rate than their production. Thus from the present observations, it can be safely concluded that during its prolonged exposure, fish appears to fall prey to chronic stress and thus TEC values observed a drastic decline till the end of the experiment. Decline in TEC by affecting the respiratory physiology of the fish can hence results in deterioration of the health status of fishes.

In corollary to TEC, Hb and Hct too have been observed to exhibit overall significant decline ($P < 0.01$) in their values in sevin treated fishes in all exposures (Table 1). Decline in Hb and Hct may be attributed to RBC lysis/destruction (Fig.) and greater prevalence of erythroblasts (immature RBCs) (Fig. 4) in the general circulation in sevin treated fishes. Availability of greater number of erythroblasts in general circulation appears to be an attempt on the part of fish to compensate for low TEC. Similar to present findings, Samparth *et al.* (1993), Musa & Omoregie (1999) and Witeska & Koscuik (2003) also held the presence of erythroblasts in blood and lysis of RBCs as the main reason for decline in Hb and Hct values in fishes following exposure to xenobiotics

Calculated Values of TEC/Red Blood Cell Indices: Red blood cell indices include mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). These are the representatives of TEC, Hb & Hct. These hematological indices fluctuate under varying ecological and biological conditions and prove to be an asset in diagnosing the structural and functional status of body/organs of fish during exposure to toxicants. These calculated indices showed significant alterations in their values with increasing concentrations of sevin in all the treated groups compared to control groups (Table 1).

MCV gives an indication of the status or size of RBCs (Alwan *et al.*, 2009). Cells of normal size are normocytic whereas those of small size are microcytic and cells which are larger in size are macrocytic. MCV exhibited significant increase ($P < 0.01$) in its values till the end of the 60 days of the experimental period (Table 1). Tyagi and Srivastava (2005), Adeyemo (2007), Gupta (2008) and Gupta *et al.* (2009) also documented increase in MCV in xenobiotic treated fishes.

Increase in MCV values, according to present author, can be attributed to reduction in TEC values because MCV and TEC have an inverse relationship. Hence decline in TEC presently have been observed to result in an increase in MCV values in sevin treated fishes. Ruparelia *et al.*, (1990) and Gupta *et al.*, (2009) upholding an association between MCV and TEC, attributed increase in MCV values to destruction of RBCs.

Increased MCV values are also suggestive of RBC swelling/macrocytosis. Nikinmaa and Huestis (1984) who observed stress induced swelling of fish RBCs attributed it to the osmotic disturbances and uptake of electrolytes and water into the cells accompanied by acidification of plasma and cytoplasm of RBC. Presently also, the blood of fishes under toxicity of sevin has been observed to depict increased prevalence of swollen RBCs (Fig. 4). The number of such RBCs was significantly well marked during second half in sevin treated fishes (Fig. 6). Swollen RBCs by increasing the volume of formed element can disturb the osmotic balance and hence may be lethal for the organism in question.

MCH and MCHC MCH represents the amount of Hb in the RBC of an organism whereas MCHC is the average concentration of Hb in given volume of blood. Both MCH and MCHC manifested appreciable alterations in sevin treated fishes (Table 1). MCHC categorizes RBC according to their Hb concentration. Cells with normal Hb are called as normochromic and those with lower than normal concentrations are known as hypochromic. Because there is no physical limit to the amount of Hb that can fit in a cell, no hypochromic category exists.

As evident from the tables 1, MCH and MCHC exhibited fluctuating pattern in sevin exposed fishes. Such changes in MCH and MCHC may be either due to the i) prevalence of large number of small sized immature RBCs (erythroblasts) in general circulation (Fig. 4) or ii) reduction in cellular blood iron thereby resulting in reduced Hb synthesis. Thus fluctuating pattern of MCH and MCHC definitely appears to be the reflection of marked decline in Hb because of chronic toxicity of sevin. Carvalho and Fernandes (2006) in *Prochilodus scrofa* and Kori-siakpere and Ubogo (2008) in *Heteroclaris* species also documented reduction in cellular blood iron to be the causative of decline in MCH and MCHC in fishes exposed to different xenobiotics.

From the above discussion on the calculated indices of RBC, it can be aptly stated that long-term exposure of fish *L. boga* to sevin produced marked effect on red blood cell indices. This simply implies that toxicity of sevin make fishes unable to sustain normal functioning of RBCs and hence may suffer from chronic anaemic conditions which ultimately affect their health status.

Presently fish *L. boga* have been diagnosed to suffer from two types of anaemias under the influence of sevin toxicity viz., Megaloblastic and hypochromic macrocytic anaemia.

Megaloblastic anaemia: It is a pathological condition in fishes which occur either due to depression in TEC, Hb and Hct or greater influx of erythroblasts or immature RBCs in the general circulation or even both. In sevin treated fish, both these factors were found to be very prominent and well marked. Exposure to sevin has been observed to induce more severe megaloblastic anaemic conditions in fishes. Tyagi and Srivastava (2005) and Gupta (2009) also reported megaloblastic anaemia in fishes following chronic exposure to xenobiotics and they attributed it to the release of large number of erythroblasts in the general circulation of treated fishes.

Hypochromic macrocytic anaemia: Decreased level of MCH and MCHC and increased values of MCV (Table 1) as observed presently are indicative of hypochromic macrocytic anaemia in *Labeo boga* under chronic exposure to sevin. Occurrence of hypochromic microcytic anaemia in fish upon exposure to xenobiotics has been reported by Arnaudova *et al.* (2008) who attributed such anaemia to decreased MCHC and MCV in fish. Presently, however, an increase in cell size was observed contrary to decline referred by the above cited worker. Therefore, the anaemia has been designated to be hypochromic but macrocytic rather than microcytic anaemia. However, similar to present findings Palanisamy *et al.* (2011) also diagnosed hypochromic macrocytic anaemic condition in fish *Channa striata* as a result of stress of xenobiotics.

Thus, it can be safely stated that long-term exposure of zinc and sevin may lead to production of anaemic conditions in fish *L. boga*. Such conditions definitely deteriorate the health status of fish ultimately affecting its growth and quality.

Leucocytes or White blood cells (WBC): Leucocytes play an important role in the regulation of immune system. Leucocytes, presently were found to depict a significant decline ($P < 0.01$) till the end of 60 days of experimental period (Table 1).

Leucocytes in fish respond to various stressors including infectious and chemical irritants (Christensen *et al.*, 1978 and Kori-siakpere and Ubogo, 2008). Hence their increase or decrease is a normal reaction on the part of an organism in a bid to cope up the stress. Leucocyte demonstrates that the immune system does get influenced under toxic conditions.

As evident from DLC (Table 2) it has been observed that both agranulocytes (lymphocytes & monocytes) and granulocytes (neutrophils, eosinophils and basophils) witnessed significant decline $P < 0.01$ in sevin treated fishes (Figs. 4, 5 & 6).

Lymphocytes, which accounts for major contribution to TLC, are the blood cell constituents usually responsible for specific type of immune response. However, in sevin treated fishes, lymphocytes showed decline in their number till the end of the experimental duration. Such drastic decline in lymphocytes reflects that chronic exposure of sevin might have resulted in inhibition of lymphopoiesis thereby affecting the immune functioning of fishes under stress.

In sevin treated fishes, monocytes and rest of WBCs i.e., neutrophils, eosinophils and basophils depicted decline (Fig.4) throughout the experimental period of 60 days. All these WBCs are phagocytic in action (Anderson, 2003) and their decline implies that the phagocytic activity of the sevin treated fishes gets almost totally hampered.

During the course of present studies, another interesting observation worth mentioning is that macrophages make their appearance in general circulation (Fig.6) in sevin treated fishes. Macrophages otherwise are not the normal constituents of blood but get surfaced in general circulation only under the stressful conditions to enhance the phagocytic activity of the blood components (Anderson, 2003). Present author proposes that on the basis of appearance of macrophages (which are known to be phagocytic) in sevin treated fishes (Fig.6), when otherwise, RBCs were declining, it appears that macrophages simply strengthen the phagocytic activity of fish for getting rid off of debris produced by breakdown of RBCs under stress of sevin.

From the above discussion, it can be deduced that sevin appears very deleterious to WBC. The fish succumbs to the chronic stressful conditions caused by sevin toxicity and its immune system gets totally paralyzed

thereby resulting in drastic decline in TLC in the general circulation. Moreover, such fishes can become easily susceptible to diseases and infections thereby affecting their health status.

Thrombocytes: Thrombocytes in corollary to mammalian blood platelets play an important role in blood clotting (Atamanalp *et al.*, 2010) and prevents blood loss from hemorrhaging. Thrombocytes are also known to be phagocytic in action (Anderson, 2003). Presently, thrombocytes were found to depicted a significant increase ($P<0.01$) in all exposures (Tables 2 and Figs. 4, 5 & 6).

Under prolonged/chronic sevin exposure, the fishes would have undergone severe degeneration and total loss of cellular architecture of haemopoietic organs ultimately causing tissue damage and injury which can even lead to blood leakage. Increase in thrombocytes in sevin treated fishes (Fig. 5), it may be mentioned can cause arrest of any sort of internal bleeding. Atamanalp *et al.* (2010) and Kayode & Shamusideen (2010) also held similar viewpoint for increase in number of thrombocytes in fish under stress. Moreover, thrombocytes being phagocytic in function, their increased number may contribute in the strengthening of the phagocytic machinery of the fish under sevin toxicity.

Conclusion: Analysis of haematological data in sevin intoxicated fishes suggests that changes in haematological parameters can effectively be used as sensitive indicators of both pollutional stress as well as to compare the toxicity status of different xenobiotics (presently carbamate pesticide, sevin). Sevin even at sublethal concentrations appeared to cause qualitative as well as quantitative changes in the haematological parameters of fish *L. boga*. Sevin caused drastic decline in both RBC and WBC dependent parameters of fish thereby depicting the higher toxicity level of sevin. Moreover, exposure to sevin for prolonged period produced anaemic effects in fishes (being more chronic/severe in higher sub-lethal concentration groups of sevin exposed fishes) thereby deteriorating its nutritive value.

It is therefore strongly recommended that agricultural discharge (the main source of sevin) should be properly treated before disposing their runoff in the aquatic ecosystem otherwise such xenobiotics when introduced in excess into the aquatic ecosystems are capable of affecting the aquatic life negatively.

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Table 1: Haematological parameters of *Labeo boga* (Mean \pm S.D.) exposed to different sublethal concentrations of sevin.

Parameters	Control	20% (Group I)	40% (Group II)	60% (Group III)
TEC ($\times 10^6$ cm/mm ³)	2.70 \pm 0.15	1.80 \pm 0.99	1.60 \pm 0.56	1.30 \pm 0.48
Haemoglobin (gm/dl)	6.4 \pm 0.72	5.1 \pm 0.19	4.2 \pm 0.99	4.0 \pm 0.67
Haematocrit (%)	35.0 \pm 0.16	25.66 \pm 0.79	23.44 \pm 0.56	20.21 \pm 0.47
MCV (fl)	125.0 \pm 0.79	134.81 \pm 0.45	139.21 \pm 0.45	141.92 \pm 0.88
MCH (pg)	22.86 \pm 0.56	25.53 \pm 0.37	24.02 \pm 0.24	21.99 \pm 0.47
MCHC (%)	18.28 \pm 0.87	16.42 \pm 0.56	17.31 \pm 0.59	17.54 \pm 0.99
TLC ($\times 10^6$ cm/mm ³)	10.0 \pm 0.55	9.17 \pm 0.99	8.39 \pm 0.57	7.85 \pm 0.58

Table 2: Differential Leucocyte Count of *Labeo boga* (Mean \pm S.D.) exposed to different sublethal concentrations of sevin.

Parameters	Control	20% (Group I)	40% (Group II)	60% (Group III)
Lymphocytes (%)	23.73 \pm 0.66	18.18 \pm 0.56	17.37 \pm 0.70	16.08 \pm 0.68
Monocytes (%)	14.94 \pm 0.98	12.86 \pm 0.69	12.34 \pm 0.54	11.48 \pm 0.49
Neutrophils (%)	19.40 \pm 0.77	11.80 \pm 0.57	11.17 \pm 0.86	10.08 \pm 0.77
Eosinophils (%)	7.46 \pm 0.19	4.74 \pm 0.77	4.46 \pm 0.18	4.23 \pm 0.99
Basophils (%)	5.97 \pm 0.56	4.67 \pm 0.57	4.35 \pm 0.68	3.79 \pm 0.69
Thrombocytes (%)	28.50 \pm 0.88	47.30 \pm 0.79	49.49 \pm 0.35	55.22 \pm 0.88

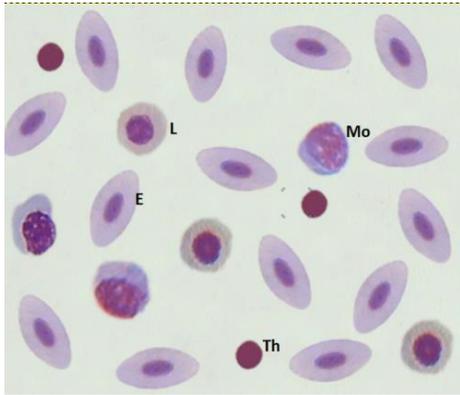


Fig.1 Microphotograph of blood smear from control fish *Labeo boga* showing Erythrocytes (E), Monocytes (Mo), Lymphocytes (L) & Thrombocytes (Th) (100x)

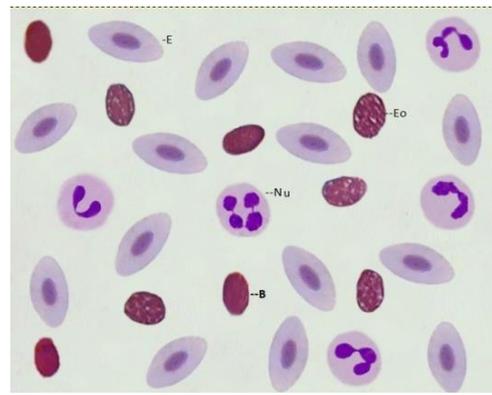


Fig.2 Microphotograph of blood smear from control fish *Labeo boga* showing Neutrophils (Nu), Eosinophils (Eo) & Basophils (B) (100x)

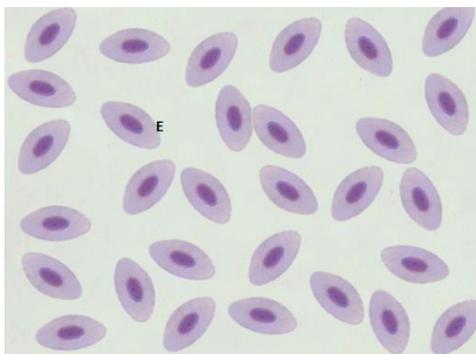


Fig.3 Microphotograph of blood smear from control fish *Labeo boga* showing Erythrocytes (E) (100x).

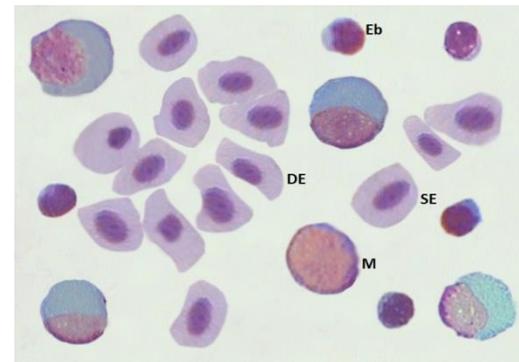


Fig.4 Microphotograph of blood smear from sevin treated fish showing Distorted Erythrocytes (DE), Swelled Erythrocytes (SE) and prevalence of Erythroblasts (Eb) (100x)

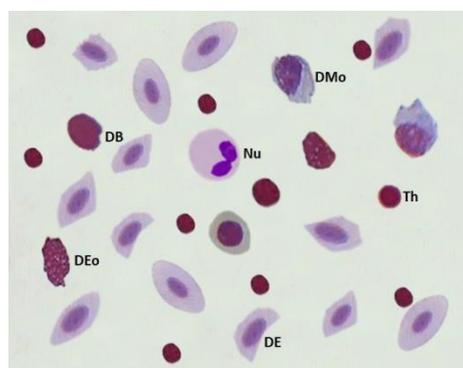


Fig.5 Microphotograph of blood smear from sevin treated fish showing Distorted Erythrocytes (DE), decrease in lymphocytes (L), Distorted Eosinophils (DEo), Distorted Basophils (DB) With increase in Thrombocytes (Th) (100x)

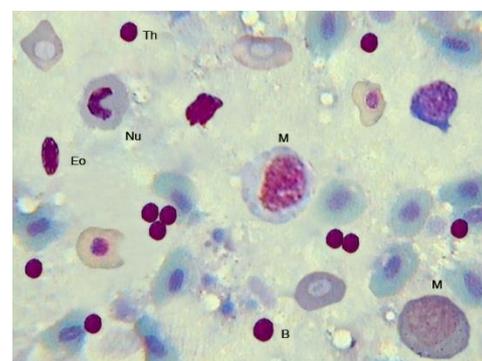


Fig.6 Microphotograph of blood smear from sevin treated fish showing prevalence of Macrophages (M) (100x).