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**Research Article**

# Methyl Parathion Insecticide Induced Morphological and Behavioural and Haematological Changes in the Freshwater Fish, *H. Molitrix* (Silver Carp)

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**Abstract:** The present study was conducted to determine the effects of Methyl parathion on morphological behavioural and some haematological indices in *Hypophthalmichthys molitrix* (Silver carp). The Silver carp were exposed to three different concentrations of (1, 5 and 10 ppm of the insecticide) for four days. The morphological and behavioural changes were observed and recorded. The values of erythrocytes (RBC- 1.65 ± 0.132), packed cell volume (PCV- 30.55±0.105) and hemoglobin (Hb-11.60±0.056) and haematocrit (Ht-0.64±0.340) value were significantly (P<0.005) decreased as compared to control (4.63±0.240, 48.01 ±0.540, 18.1±0.320 and 2.6±0.46) respectively. The values of mean corpuscular emoglobin content (MCHC-45.76±0.31) and mean corpuscular hemoglobin (MCH 38.30±0.127) were significantly (P<0.05) increased whereas, the mean corpuscular volume (MCV-90.50±0.158) was found to decrease with Methyl parathion administration in comparison with control (34.02±0.33, 31.13±0.076, 94.31±0.489). Lymphocyte (P<0.05), Neutrophils, Eosinophil, and Basophil increased significantly (P<0.005). In conclusion, the changes in blood parameters of the fish exposed to Methyl parathion may be related to decreased immune system. Assessment of haematological parameters could provide a useful indicator of Methyl parathion toxicity in freshwater fish.

**Keywords:** Effect; Methyl parathion; H. molitrix; morphological, behavioural Haematological parameter.



## INTRODUCTION

The rapid increase in Human population has led to the continuous exploitation and contamination of aquatic environment by several toxic chemical components from industrial, agricultural and domestic activities. For insect pests' management in agricultural fields and control of vectors causing human disease, a variety of pesticides and insecticides are used in several states in India. These chemicals enter into the rivers systems and aquaculture ponds through runoff from treated areas by contaminated rivers (Austin, 1999 and Goel, 2000). Wherever these harmful chemicals are dumped or released into water bodies, they affect the aquatic fauna in two ways. These chemicals not only alter the habitat of many aquatic organisms but also lead heavy mortalities of all aquatic fauna in concentration beyond permissible limits. In addition in lower concentration, these chemicals lead to bioaccumulation and ultimately enter into food chain to human beings that cause long-term effects on human health (Austin, 1999; Goel, 2000; Xie *et al.*, 1996 and Suresh *et al.*, 2013).

Aquatic contaminations of pesticides cause acute and chronic poisoning of fish and other organisms. Pesticides damage vital organs and skeletal system and also cause behavioural changes of the exposed fishes (Omitoyin *et al*, 2006; Johal *et al*, 2007; Velmurugan *et al*, 2007).

Konar (1975) and Basak and Konar (1997) reported that even though the pesticides has some effective results for its immediate action as pest control but the adjacent water bodies of the agro-farms get contaminated by the residual release and further indicated that such pesticides act as the catalyst for gradual deterioration of aquatic ecosystem. Hossain (2002) reported that the high degrees of pesticides concentration have deleterious effect on the morphology and behavior of fish along with changes in its growth and reproduction (M cKim *et al.*, 1975). The

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long-term effect of pollution showed significant variation ($p<0.05$) in the expression of Myo D and Glut 4 protein by Suresh *et al.,* (2013). These molecular changes are expected due to the influence of various pollutants in the Ennore Creek water.

Huculeci *et al.,* (2009) indicated that even the low concentration of Malathion, harms fish in numerous ways by altering the growth parameters, reducing the hematological properties, swimming ability and also depleting some of the biochemical parameters (Glycogen, cholesterol and total protein). Studies of Sivachandran and Mazher Sultana (2014) and Mazher Sultana and Suresh (2014) also reported that some vital organs *viz.*, kidney, liver, gills, stomach, brain, muscles, reproductive organs and DNA of fishes was found to be affected by aquatic pollutants.

Since insecticidal toxicity is harmful to physiological activities of the aquatic organisms like fishes, it is essential to investigate the effects on behavior changes and haematological properties. Therefore, to understand the toxic effect of Methyl parathion on morphological, behavioural and haematological properties of the indigenous freshwater fish *Hypophthalmichthys molitrix* was undertaken in the present study.

## MATERIALS AND METHODS

Healthy ***H. molitrix***, of both sexes with relatively same size ranging from (10–15 cm) and weight about (25–50 gm), oxygen packed in polythene bags were collected from culture ponds of Bharath Fish Farm, Poondi, Thiruvallur district, Tamil Nadu; were brought to the laboratory with minimal stress and released very carefully into the fish tanks half filled with bore well water. They were maintained in the stocking tank and acclimatized before experimentation.

The fishes were fed daily with pelleted feed at 5% body weight, twice (morning and evening) prepared by sieved rice bran, pounded groundnut oil cake, tapioca powder and mineral mixture. The feeding was stopped one day prior to experiment.

**Maintenance of Fish**

The fishes were maintained in the aquarium tanks of size 1'l x 2'b x 1'h throughout the period of study. Potassium permanganate (0.02%) was used as disinfectant to clean the tanks before and after experiments. The tanks were filled with water (2 litres per fish) and covered with mesh cloth to prevent the mosquitoes breeding in the water and also to prevent the fishes from jumping out of tank. During the period of study the room temperature fluctuated from 29˚C to 32˚C. The dissolved oxygen content of water used for the study was 4.8 to 5.4 ml / litre and salinity of 0.82 - 0.85 ppm. The pH of water was in the range of 7.2 - 7.4. Fish were exposed to concentrations of 1, 5 and 10 ppm of Methyl parathion for 12, 24, 48 and 96 hrs (Ashaduzzaman *et al.,* 2016). Temperature, dissolved oxygen, conductivity and pH were measured during the experiment (APHA, 2005).

For this, three treatment groups including control group were created to test toxicity; each treatment was replicated thrice with 10 fish per tank with 60 L water capacity. Fishes were exposed to Methyl parathion at a nominal concentration of 1, 5 and 10 ppm for four days toxicity testing (Ashaduzzaman, 2016). Fish deaths were saved (24, 48, 72 and 96 hrs) after the beginning and died fishes were taken instantly from the tank (Banaee *et al.,* 2011).

**Blood samplings and Hematological assay**

After the test period, fishes were taken out of the water rapidly and held securely in a loom with a soft cloth covering the head for taking blood samples. Blood samples was collected from the tail blood vessel by heparinized syringes and immediately stored on ice and the blood parameters computation were carried out on fresh blood. Blood erythrocytes (RBC $x10^4mm^{-3}$) and leukocytes cells ($10^6mm^{-3}$) counting were performed by diluting heparinized blood with Giemsa stain at 1:30 dilution using a hemacytometer Neubauer chamber under the light microscope. The leukocyte differential calculation (%) was performed in peripheral blood spots stained by Merck Giemsa, giving the Neutrophils quantity of differential neutrophils and the mononuclear quantity of differential lymphocytes, eosinophile and monocyte.

The other haematological parameters examined were haemoglobin (Hb, g/dL), hematocrit (Hct, %), mean corpuscular haemoglobin (MCH= Hb in g/ RBC in millions × 10pg), mean corpuscular volume (MCV= packed cell volume as percentage/RBC in millions× $10^{i3}$,fl), mean corpuscular haemoglobin concentration (MCHC= Hb in g/packed cell volume× 100 g per 100 mL,%), and leukocyte count ($WBC×10^4/mm^3$) (Shamoushaki *et al.,* 2012; Klont *et al.,* 1994).

**Statistical Analysis:**

The variations through the medium of (±SEM) between groups were evaluated using Independent Samples-t test. 95% confidence limits were considered important. SPSS 15.0 software was used for data analysis (Karatas, 2015).

## RESULTS AND DISCUSSION

Morphological changes were observed in the fishes treated with Methyl parathions. The remarkable variation was appearance of black spots over the body surface, the general color become dull and less shiny. Liberation of large amount of mucous and excreta from the Methyl parathion treated fish were observed as compared with the control. The affected fish revealed variety of behavioral changes like, drifting up and down frequently with widely opened mouth, erratic movement of the operculum and resting flat at the bottom of the aquaria. Halappa and David (2009) also

reported similar changes in *Cyprinus carpio* (Lin) following sublethal exposure to chloropyrifos.

Behavioural changes have been observed due to bioaccumulation of xenobiotics in aquatic species cause major threat to aquatic life. Many fish species show uptake and accumulation of many contaminants such as pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons and heavy metals (Heger *et al*, 1995 and Pandey *et al*, 2009).

Mazher Sultana *et al.*, (2014) also observed similar behavioural manifestation of acute toxicity like copious secretion of the mucus, loss of scales, discoloration, surfacing and darting movement in C. *marulius* exposed to sub lethal concentration of the kerosene at 24 to 96 hrs. After 72 hrs exposure, the fishes exhibited lethargy and erratic movements suggesting loss of equilibrium at high concentration. At the time of death transient hyper activity was also observed in *Rasbora daniconius* on exposure to tannery effluents (Mazher Sultana and Saraswathy, 2012).

Haematological parameters constitute as an important biological system for the survival of fishes against diseases. Summarwar (2012) indicated that aquatic ecosystem contaminated by insecticides has detrimental effect on haematological parameters of fish. Carbofuran was observed to cause the reduction in the hemoglobin value of teleost fishes by Singh and Srivastava (2010). Similarly Far *et al.*, (2012) reported a significant decrease in the Haemoglobin content in diazinon exposed fish (rain-bow trout). A significant decrease in haemoglobin content was recorded in *Cyprinus carpio* (Adedeji *et al.*, 2009). In the *Orecochromis niloticus* a marked decrease in Haemoglobin content was also observed due to Dimethoate and Malathion exposure in the same species (Sweilum, 2006).

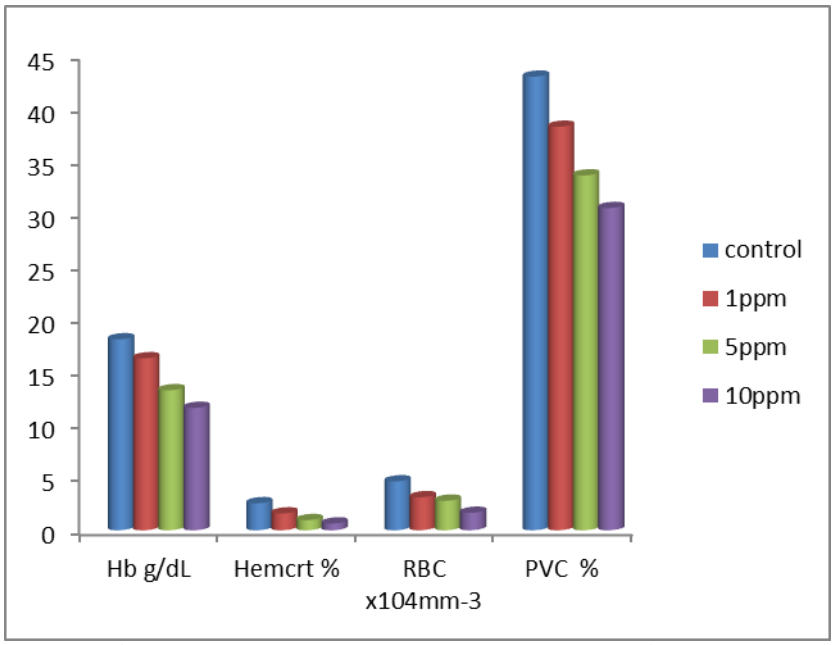


**Fig 1:** Haematological response of *H. molitrix* exposed to Methyl parathion (1, 5 and 10 ppm) after 96 hrs and in control fish (Mean ±SD)

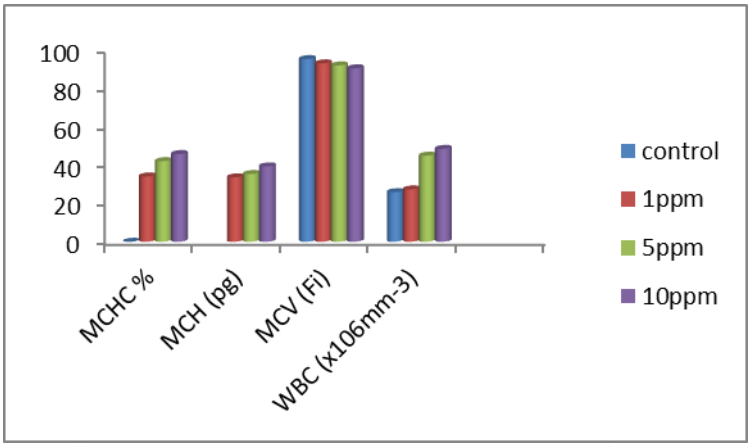


**Fig 2:** Haematological response of *H. molitrix* exposed to Methyl parathion (1, 5 and 10 ppm) after 96 hrs and in control fish (Mean ±SD)

The present study also found a significant reduction in the levels of RBC, PCV, Haemoglobin (p=0.005) and Ht content of ***H. molitrix*** due to the application of Methyl parathion parathion as compared to control group of fish (Fig 1). Similar results were observed in *O. mykiss* exposed to Cypermethrin (Atamanalp *et al.*, 2002), and to Diazinon (Tayfun and Mevlut, 2018). The number of RBC,WBC, Hb content and Ht amount decreased significantly in Phe-exposed fish compared to control in all sampling d ($p<0.05$) by Mehrnaz *et al.,* (2018).The values of Mean Corpuscular Hemoglobin content (MCHC) and Mean Corpuscular Hemoglobin (MCH) were significantly ($P<0.05$) increased with Methyl parathion (Fig 2) similar to diazinon administration in *O. mykiss* (Tayfun and Mevlut, 2018) but the Mean Corpuscular volume (MCV) value was observed to decrease statistically insignificant ($P>0.05$). Haematocrit was observed to decrease significantly in *Acanthopagrus latus* due to phenanthrene exposure (Mehrnaz *et al.,* 2018) as well as in *H. molitrix* in the present investigation.

A reduction in leucocyte count was observed in *C. punctatus* after chronic exposure of freshwater teleosts to monotrophos (Singh *et al.,* 1992). A significant decrease in leucocyte count was found due to the exposure of *Cyprinus carpio* to toxic environment of Diazinon (Banaee *et al.*, 2008). In the present study the leucocyte count was found to increase significantly (p=0.005). However, a significant rise in leucocyte content was reported in *C. punctatus* due to toxic effects of Malathion (Magar and Duve, 2012).

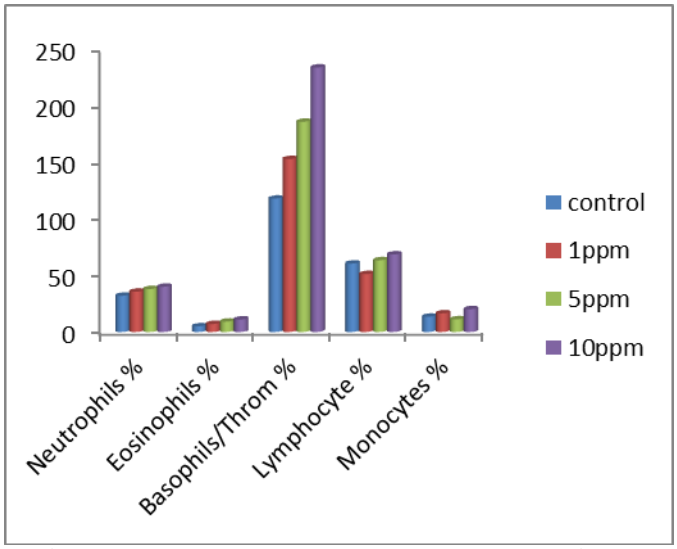


**Fig 3:** Haematological response of *H. molitrix* exposed to Methyl parathion (1, 5 and 10 ppm) after 96 h and in control fish (Mean ±SD)

The lymphocytes, monocytes, basophils, eosinophils and neutrophils (p=0.005) was observed to increase significantly at 1, 5 and 10 ppm of Methyl parathion after 12, 24, 48 and 96 hrs exposures (Fig 3). This increase in the number of neutrophils, might be due to increase in phagocytosis of damaged cells. Hedayati (2012) indicated that Lymphopenia, a decrease in the number of lymphocytes, might presumably resulted from the disintegration of the cell membrane.

A significant decline in lymphocyte count and a marked increase in neutrophil in *C. carpio* when exposed to Diazinon has been recorded (Velisek *et al.*, 2009). The number of lymphocyte was significantly decreased and neutrophil was increased (Saeedi and Singh, 2013) but our observations showed increase in lymphocyte and neutrophil content in *H. molitrix.* Thakur and Pandey (1990) observed a significant increase in neutrophil content due to toxic stress induced by the pulp mill effluent. Monocrotophos decreased neutrophil count whereas increased the number of lymphocytes in freshwater fish, *Channa punctatus* (Agrahari *et al.*, 2006). The reductions in lymphocyte and rise in neutrophil content could be due to the destruction of haematopoetic tissue and decrease in non-specific immune system due to increased concentration of defensive poison.

Banerjee *et al.,* (2003) and Agarwal *et al.,* (2006) reported reduction in monocyte content in *C. punctatus* on exposure to Rayon industry effluents and Monocrotophos. But the present study recorded enhancement in monocyte content in *H. molitrix.* Ghosh and Banerjee, (1993) also revealed a significant rise in the percentage of monocytes in *H. fossilis* on exposure to sublethal concentrations of synthetic pesticide, Dimethoate.

Toxicity of Methyl parathion enhanced the number of eosinophils in *H. molitrix* which is in consistent with the study of Diazinon toxicity in *Cyprinus carpio* by Svobodova *et al.*, (2001). In *H. molitrix* the number of eosinophils and basophils was found to increase significantly but Sharma and Gupta (1982) reported decrease in basophils and increase in eosinophils content of *C. batrachus* to carbon tetrachloride exposure.

Hedayathi and Hassan (2015) reported in their study that in *H. molitrix*, the values of leukocytes (WBC), haematocrit (Ht), hemoglobin (Hb), MCHC, lymphocyte, cortisol and glucose were significantly increased ($P < 0.05$) as well as the values of MCV and MCH were also increased significantly at 48 hrs and then decreased at 96 hrs ($P < 0.05$). They observed a significant increase in neutrophils count at 48 h and later a significant decrease at 96 hrs ($P < 0.05$). There were no significant differences in RBC, monocyte and eosinophile counts among treatment group. Our results are not in conformity with the above reports. In the present investigation there is a significant decrease in Hb, Ht, RBC, MCV and a significant increase in MCHC, MCH, WBC, Neutrophils, Lymphocyte, Monocyte, Eosinophil and Basophils (Fig 1, 2 &3).

However, it was also reported by Singh and Srivastava (2010) that the contents of haemoglobin, leucocytes, lymphocsytes and thrombocytes were reduced whereas neutrophils, eosinophils and ESR contents were increased due to the effects of carbofuran and carbaryl. Our results are in close conformity with those reported above. However, more comprehensive works are to be solicited.

# CONCLUSIONS

The detrimental effects of Methyl parathion are very much reflected in the significant alterations in various haematological parameters of *H. molitrix*. The blood parameters were observed sensitive to different prospect of Methyl parathion exposure. This suggests that the environment must be protected against any toxicity produced by such chemical agents. More knowledge of these activities in fish is necessary before they can be employed as biomarkers of stress due to pollution.

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**Conflicts of Interest**

The authors have no conflicts of interest to declare.

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