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Research Article

HEMATOLOGICAL RESPONSE IN A FRESHWATER FISH *CHANNA STRIATUS* EXPOSED TO METHOMYL PESTICIDE

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ABSTRACT

In the present study of sub lethal concentrations of methomyl pesticide administered to freshwater fish, *Channa striatus* for a 96 hours of methomyl exposure 0.54 ppm based on the period of(10% and 30%)15 and 30 days about significantly (P>0.05) hematological parameters alterations. The hematological analysis showed significantly (P>0.05) reduction in red blood cells (RBC) count, hemoglobin (Hb) value, packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular (MCV). The parameters such as total white blood cells (WBC) increased. The study has thus indicated marked changes in blood of *C. striatus* after exposure to methomyl pesticide.

Keywords: Methomyl, *Channa striatus,* Hematology, Sub lethal toxicity, Hemoglobin.

INTRODUCTION

Adversely human activities are directly or indirectly affect the environment (Tamizhazhagan & Pugazhendy, 2016). Methomyl is a highly toxic carbamate insecticide, except their effects are more reversible and less severe. The most popular of these pesticides for residential uses is carbaryl and propoxur. The heavy metal and pesticide contamination of aquatic system has attracted and attention of researchers to all over the world and has increased in the last decades due to extensive use of them in agricultural, chemical and industrial processes (Padmapriya *et al*., 2017). Many carbamates such as methomyl and aldicarb are also used in agricultural applications. Carbaryl exposure has been estimated based upon urinary measurements of 1-naphthol, its most abundant metabolite. The organophosphates that cause the most illness in agricultural workers are the high toxicity compounds mevinphos, methomyl, methamidophos, oxydemeton, and parathion, as well as the moderate toxicity compounds dimethoate and phosalone, with less agricultural use of some of these compounds in recent years in the United States. Carbamate pesticides typically cause less severe and shorter duration toxicity compared with organophosphates due to a more reversible complex formation between the carbamate and the cholinesterase enzyme (Tamimi *et al*., 2008). Organophosphate pesticides also inhibit this enzyme, although irreversibly and cause a more severe form of cholinergic poisoning. Environmental pollution occurs when the environmental degradation crosses limit so that. It becomes lethal to living organisms (Usha *et al*., 2017). Today environmental pollution has become not only a national but also an international problem. Indiscriminate and extensive use of insecticides to protect crops possesses a serious threat to humans and the surrounding environment (Padmapriya *et al*., 2017). Heavy metal constitutes a serious type of pollution in fresh water and being stable compounds (Pichaimani *et al*., 2017).

Environment is the sum total of water, air and land interrelationships among themselves and also with the human being, other living organisms and other property (Usha *et al.*, 2017 b) Aquatic ecosystems that run through agricultural areas have high probability of being contaminated by runoff and ground water leaching by a variety of chemicals. Highly effective pesticides are used tremendously, which on entering the aquatic environment bring multiple changes in organism by altering the growth

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rare, nutritional value, behavioral pattern, etc. A major part of the world's food is being supplied from fish source, so it is essential to secure the health of fishes (Tripathi *et al*., 2002). These pesticides are non-biodegradable and accumulate in the food chain. Mostly they are prone to affect the nervous system causing tumors in living organisms.

Pesticide pollution affects aquatic organisms through food chain including human beings (Dutta & Maxwell, 2003). These insecticides by their easy entry into the aquatic ecosystem (as runoff) may also result in damage of non-target organism particularly fishes. Determination of extent of damage to different body systems viz., respiration, feeding, osmoregulation and reproduction including blood (du Preez & Van Vuren, 1992) exposed to different xenobiotics therefore become very important. Among different systems haematology act as an essential index of the general health status of the fish (Larsson *et al*., 1985). Lindane is commonly used insecticide in crop field and its bioaccumulation is known to cause impairment in various physiological processes under the conditions of long term exposure.

The assessment of the ecotoxicological risks caused by pesticides to ecosystems is based on toxicity data and the effects of pesticide preparations on non-target organisms. Fish are among the group of non-target aquatic organisms. Blood parameters are considered pathological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari *et al*., 2004). It was reported that the blood parameters of diagnostic importance are erythrocyte and leucocytes counts, haemoglobin, haematocrit and leucocyte differential counts would readily respond to incidental factor such as physical stress and environment stress due to water contaminants (Bhatnagar *et al*., 1992; Railo *et al*., 1985). Some authors (Agarwal & Chaturvedi, 1995; Chauhan, Saxena, & Kumar, 1994; Reddy & Bashamohideen, 1989) have reported a decrease in hematocrit, hemoglobin and red blood cells values of some fish after their exposure to insecticides. Haematological parameters reflect the state of fish under stress more quickly than other commonly measured parameters, as they respond quickly to changes in environmental conditions (Alkinson & Judd, 1978). For monitoring stress responses, predicting systematic relationships and the physiological adaptations of animals, haematological parameters have been widely used for the description of general health of fish (Blaxhall, 1972).

MATERIALS AND METHODS

Fish collection and laboratory conditions

The freshwater healthy fish *Channa striatus* of the weight (22.34 ± 0.79 g) and length (17 to 20 cm) were selected for the experiment and were collected from ponds in around Thanjavur. Fish was screened for any pathogenic infections. A Glass aquarium was washed with 1% KMnO4 to avoid fungal contamination and then sun dried. The fishes were maintained in 300 L tank containing dechlorinated tap water (Temperature 26°C; total hardness- 516 ± 22mg/L; Do-5.7 ± 70.2mg/L; salinity- 1.3 ± 0.13 ppm and PH- 7.8 ± 0.03). Fish was acclimated to laboratory conditions for 15 to 30 days prior to experimentation. They were regularly fed with commercial food and the medium (tap water) was changed daily to remove fasces and food remnants.

Chemical

The insecticide used in this experiment was Methomyl 40% W/W (Reg. No. CIR. 31, 760/99/Methomyl (SP)-71) were purchased from Thanjavur, Tamil Nadu, India. The Methomyl insecticide was used only for the present experiment.

Experimental design of Sub-lethal concentration

The experimental group was vulnerable to a sub lethal concentration of the insecticide (0.54 ppm L^{-1}) during 15 and 30 days. Toxicity tests carried out in accordance with in standard methods (APHA, 1800). Based on acute toxicity test (96h LC50) sub-lethal concentrations (10% and 30% of 15 & 30 days) were derived for arsenic which served as the experimental concentration of the arsenic in the subsequent experiments. Ten fish were exposed to each concentration for a period of 15 and 30 days. Control batch was maintained simultaneously.

Fish dissection and preservation

The fishes were divided into a four group, each group of ten healthy fishes were transferred to plastic tough having capacity of 10 litres and they exposed to 10% and 30% sub lethal concentration of methomyl (0.054ppm). One group was kept as control. At the end of exposure period the blood was collected in glass tube by cutting the caudal peduncle, using EDTA as anticoagulant. Maximum 2ml blood was taken for hematological studies. The hematological parameters such as RBC, WBC, and Hb, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were estimated by using the methods described by the RBC and WBC were counted by haemocytometer, Hb was determined by Sahil's haemoglobinometer and PCV was measured by using Wintrobe's tube. The Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration were calculated by equation.

Mean Corpuscular Hemoglobin (MCH)

This indicates the weight of hemoglobin in a single red blood cell and is expressed in pictograms (pg) (1 pg=10^{-12}g).

$$\mathrm{MCH} = \frac{\text{Hemoglobin (g per 100 ml)}}{\text{RBC count million per cu. mm}} \times 10$$

Values were expressed as pictograms (pg)

Mean Corpuscular Hemoglobin concentration (MCHC)

This denotes the hemoglobin concentration per 100 mi of packed red blood cells and is realed to the colour of the red cells. This is expressed as percentage of packed cells.

$$\mathrm{MCHC} = \frac{\text{Haemoglobin (g/d1)}}{\text{PCV\%}} \times 100$$

Values were expressed as %

The Mean Corpuscular Volume (MCV)

This is expressed as the volume in cubic microns or femto liters of an average red blood cell.

$$\mathrm{MCV} = \frac{\text{PCV\%}}{\text{Red blood cells in millions per cu. mm}} \times 10$$

Values were expressed as femto liters.

RESULTS AND DISCUSSION

The quantitative changes of hematological parameters like, RBC, WBC, Hb, PCV, MCH, MCHC, MCV and the pathological changes of the blood cells in the fish *C.striatus* both in control and sub lethal concentration of methomyl 15 and 30 days exposed given in Table 1. Reported that significant decrease in Hb concentration after exposure to 5% malathion, may be due to impaired oxygen supply to various tissues, resulting in slow metabolic rate and low energy production (Ahmad *et al*.,1995), or may be due to increased in metabolic rate, which may have led to decrease Hb concentration level (Reddy & Bashamohideen, 1989). Decreased Hb concentration level in various fishes exposed to insecticides was reported, viz. *Heteropneustis fossilis* exposed to deltamethrin (Kumar *et al*.,1999); *Channa punctatus* to malathion (Pandey *et al*., 1981) and *Anabas testudineus* to metasystox (Natrajan, 1981). Reported that the reduction of oxygen-carrying capacity in fish may be associated with a decrease in hemoglobin concentration that is affected by deltamethrin this chemical compound appears to interfere with the ability to bind hemoglobin to oxygen during respiration (Atamanalp *et al*., 2010) this result is supported by (Maxwell).

RBC and Hb decreased in cold temperature due to mechanism in which compensate for poor oxygen uptake in prevailing hypoxic condition. Cold temperature increases the oxygen requirement (Julian *et al*.,1989). Water temperature decrease, the osmotic concentration and blood viscosity increase. Decrease in hemoglobin could be result of blood osmo concentration, as shown by an increase in plasma osmolarity. HB level connected with number of RBC. When the number of RBC decreased, HB level decreased. When temperature decreased number of WBC increased. Decreasing trend of HB during the cold temperature has been reported in many. The random use of different pesticides often causes lot of damage on non-target organism. Organophosphate pesticides constitute a large proportion of the total synthetic chemicals employed for the control of pests in the field of agriculture, veterinary practices and public health (Padmapriya *et al*., 2017).

An increase in lymphocytes suggests that the immune mechanism of fish get stimulates and become adapted under insecticide stress to fight against the pollutants in the environment. Higher white blood cell count indicates damage due to infection of body tissue may be physical stress and leukemia. Increased in the in the number of leucocytes occurs due to The decrease in the above parameters indicates stage of fish caused due to decreased erythropoetic activity or increased destruction of red blood cells further support in favors of this comes from the decrease noted PCV. Decrease in Hb indicates poor O2 transport by blood caused by damage or due to increased accumulation of CO_2 in blood. In the present investigation significant change was recorded in the RBC, WBC, Hb, MCV, PCV and MCHC. The reduction in this values might be due to spleen concentration after have been detected in fish. Cells released from spleen which is an erythropoetic organ would have lowered those values. Immunological response similarly increased in total leucocytes counts has been observed by BinuKumari & Subisha, (2010).This significant increase in total leucocytes count might be due to immunological reactions to produce more antibodies to cope with the stress induced by these toxicants.

Table 1. Hematological parameters of fresh water fish *Channa striatus* exposed to sub lethal concentrations of (10% and 30% of 96h LC_{50}) methomyl (40%) pesticide.

Parameters	Control	0.054 ppm 15 days	0.054 ppm 30 days	0.162 ppm 15 days	0.162 ppm 30 days
Hb(gm/dl)	5.96 ± 0.77^{a}	4.72 ± 0.94^{ab}	4.58 ± 0.76^{ab}	4.39 ± 0.65^{ab}	3.26 ± 0.53^{b}
WBC($\times 10^9$/L)	4.22 ± 0.80^{b}	5.29 ± 0.75^{ab}	6.70 ± 0.83^{ab}	6.43 ± 0.87	7.31 ± 1.01^{a}
RBC($\times 10^2$/L)	4.00 ± 0.53^{a}	2.76 ± 0.95^{c}	2.49 ± 0.97^{c}	3.10 ± 0.61^{b}	2.63 ± 0.51^{c}
PCV (%)	35.71 ± 0.87^{a}	28.74 ± 1.99^{b}	27.53 ± 1.84^{b}	22.50 ± 1.04^{c}	20.5 ± 0.96^{c}
MCH (pg)	25.95 ± 0.83^{a}	23.31 ± 1.48^{b}	20.16 ± 1.24^{c}	19.72 ± 0.94^{c}	16.69 ± 0.94^{d}
MCHC (%)	26.12 ± 0.83^{a}	18.14 ± 1.10^{b}	12.13 ± 0.80^{d}	15.19 ± 0.96^{c}	7.67 ± 0.98^{e}
MCV (fl)	230.24 ± 2.92^{a}	165.90 ± 16.46^{b}	162.62 ± 14.57^{c}	153.32 ± 0.87^{d}	137.88 ± 1.06^{e}

Values are given as mean ±SE. Values not sharing a common marking ([a,b,c,d,e]) different alphabets in columns differ significant at p< 0.05 (Duncan's Multiple Range Test).

Increase in the number of WBC of *C. marulius* reflects impairment of the defense mechanism and manifested in to leucocytosis to cope with such a situation. Similar results were reported in teleost by (Tyagi *et al*., 1989) and (Ramesh & Saravanan, 2008) on exposure to different pesticide. Lymphocytes are numerically predominant white blood cells in fish. Lymphocytes of fish have been regarded as immune-competent. Thus they are responsible for the production of antibodies (Ellis, 1978). Haematological parameters have been acknowledged as valuable tools for monitoring fish health, confirming maturation and monitoring any changes in the quality of water. Therefore, a change in water is one of the most important factors for fish hematology. On the basis of haematological studies, it is done to determine the sensitivity to environmental conditions and toxic substances of fish the most common cause of water pollution in developing countries is domestic and industrial waste that is directly released into streams or ponds without treatment(Tamizhazhagan & Pugazhendy, 2016). Fish are particularly sensitive to a wide verity of pesticide chemicals and toxic concentration may rise not only from spillage of agricultural practical if their use is excessive but also from several other sources(Jayalakshmi et al., 2017).

Significant decrease was observed in MCH and MCV values are similar to other studies. MCH was significantly decreased in *C. idella* after 48 h exposure to fenvalerate (Mughal, 1993). The decreased MCH and MCV levels may be a sign of hypochromic microcytic anemia (Shakoori *et al*., 1991). Residual of this pesticide alters in to the ecosystem and trouble the healthy environment and aquatic forms. Aquatic farm contains fish and other organism. But the fish is mostly affected by pesticide residuals (Tamizhazhagan *et al.*, 2017) These tests determine LC50 which is a quick estimate of different toxicants and assessment of a toxicant to estimate toxicant concentration to be used in the intermediate and long term test (Jayalakshmi et al., 2017). In contrast to our findings, there were increases in Hb and MCH values in *T. mossambica* exposed to cadmium chloride and *C. idella* exposed to sublethal doses of mercuric chloride and in *O. mykiss* exposed to cypermethrin (Atamanalp et al., 2010). RBC indices such as MCV and MCHC fell during prolonged exposure to Mercuric chloride (Mughal, 1993). The decrease in other hematological parameters is attributable to reduced MCV (Ahmad *et al*., 1995) while (Chauhan *et al*., 1994)found no significant effects in MCHC fish, *Prochilodus scrofa* on copper toxicity, that are consistent with present study regarding no significant in MCHC values.

CONCLUSION

The present investigation show that methomyl caused decrease in the hematological parameter in *Channa striatus* which propose that the methomyl pesticide may weak the impervious system and result in severe physiological problem finally to the death of fish methomyl pesticide is a used protect many fruits, vegetables and field crops a against disease, hence farmer come direct contact and may impair their health.

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