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Comparative Study on the Toxicity of Biopesticides and Synthetic Insecticide on the Biochemical and Hematological Changes of Fresh Water Fish, Nile Tilapia *Oreochromis niloticus* (L.)

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Abstract: ***Injudicious use of synthetic pesticides causes numerous adverse effects, ranging from long-term to short-lived changes in a balanced ecosystem. The impact of cassava based biopesticide (Menma) was tested along with a neem based biopesticide (Nimbecidine) and a neonicotinoid insecticide (Imidacloprid) on the fresh water teleosti, Oreochromis niloticus. The LC_{50} values of all the three insecticides were calculated and their 1/5th values were taken as the sub lethal doses. Fishes were divided into four groups and the first three were treated with Menma (0.15ppm), Nimbecidine (0.014 ppm) , Imidacloprid (0.012 ppm) and the fourth one was taken as control. Results showed that tissue protein were found decreased and the level of serum glucose and cholesterol increased in all the pesticide treated fishes. Hematological studies showed that Imidacloprid and Nimbecidine treated fishes had drastic changes in their RBCs, whereas no aberration was noticed in the Menma treated fishes. The study revealed that exposure of Menma to O. niloticus causes no significant changes in its biochemical and hematological parameters.***

Keywords: *Oreochromis niloticus*, Biopesticide, *Menma*, Nimbecidine, Imidacloprid

1. Introduction

Indiscriminate use of synthetic pesticides leads to health and environmental hazards. Fishes are more frequently exposed to such pollutants due to the runoff from the agricultural fields and that may affect the biochemical and hematological parameters of exposed fishes [1]. Realizing the consequences of synthetic pesticides to living being, globally there is a massive movement for the development of befitting alternative to synthetic pesticides. *Manihot esculenta* (Crantz), commonly known as cassava (tapioca) , is the most important calorie-producing crop grown as a staple or subsidiary food. It is also used as animal feed and as raw material for industrial products like starch, glucose, alcohol etc. The insecticidal principles isolated from cassava leaves is used for the formulation of a biopesticide named *Menma* which has widely been used against borer pests, particularly against Pseudostem weevil, *Odoiporous longicollis* in banana [2]. Banana is extensively cultivated in low land areas and the inputs applied may have a fair chance to runoff to the nearby water bodies. As there was no data available on the effect of *Menma* on fishes, the present study was undertaken and compared its effect with the commonly used neem based biopesticide, Nimbecidine and a neonicotinoid insecticide, Imidacloprid on *Oreochromis niloticus*. Nimbecidene, the derived product of *Azadirachta indica* contain the active ingredient Azadirachtsin, has responsible for its insecticidal properties [3]. Imidacloprid is known for its high selective toxicity to insects and outstanding potency as a systemic insecticide against a wide range of sucking insect pests of horticultural crops [4], [5]. The present study was aimed to determine the sub lethal effects of *Menma* compared with the neonicotinoid pesticide; Imidacloprid and the neem based biopesticides; Nimbecidine on the biochemical and hematological aspects of fresh water fish, *O.niloticus*.

2. Materials and Methods

Adults *O.niloticus* weighing 25.8 ± 1.2 g were collected from the hatchery in the district of Alappuzha, Kerala and reared for one month in a 1000L fiber tank with proper aeration and feed. Prior to the experiment, fishes were divided into four groups consisting of six fish in each group. Fish in groups 1, 2 and 3 were exposed to *Menma*, Nimbecidine and Imidacloprid in a sub lethal concentration of 0.15 ppm, 0.014 ppm and 0.012 ppm respectively in separate aquarium for a period of 14 days. The fourth group of fish kept in pesticide free water and served as the control. Temperature, pH, hardness and dissolved oxygen under laboratory condition were 27-30^{o}C, 6.2-7, 20-25mg.$L^{-1}$and 6-10mg.L^{-1}respectively and they were maintained as such throughout the experiment. The fishes were sacrificed after 14 days and subjected for the biochemical and hematological analysis.

2.1 Biochemical Analysis

Total protein was estimated according to the methods of Lowry [6]. Estimation of serum glucose was done by the method of Tietz [7] and the serum cholesterol by the method of Trinder [8].

2.2 Blood cell analysis

Blood sample taken from the caudal vein of fish was directly smeared on slides and air-dried. Smears were subsequently

fixed in methanol for 10 minutes and stained with 10% Giemsa solution for 8 minutes [9].

3. Results

Total protein were represented as µg/ml and serum glucose and cholesterol were represented as mg/dl.

3.1 Protein

Total protein decreased in gill, liver and kidney of fish *O. niloticus* exposed to *Menma*, Nimbecidine and Imidacloprid (Fig.1) and the maximum reduced level of protein were observed in the kidney tissues. In all the tissues, *Menma* treated groups exhibited almost similar values of protein as the control ones.

3.2 Serum glucose and Cholesterol

Serum glucose and cholesterol increased in all the pesticide treated groups except in Imidacloprid treated ones, where slight decrease of serum glucose were observed (Fig.2).

3.3 Blood cells

From the blood cell analysis, it was observed that the RBC of the control fish was elliptical, centrally located nucleus with thick non-granulated cytoplasm. WBC was spheroid shaped cells with centrally placed large sized round nucleus and distinct cell wall with clear cytoplasm. Remarkable changes were noticed in the Nimbecidine (Fig.3C) and Imidacloprid (Fig.3D) treated fishes such as anisopoikulocytic cells (APCs), pyknotic (PK) nucleus, nuclear hypertrophy (HY), projected and vacuolated cytoplasm in RBCs and WBCs. But the *Menma* (Fig.3B) treated fishes showed similar morphology as the control fishes.

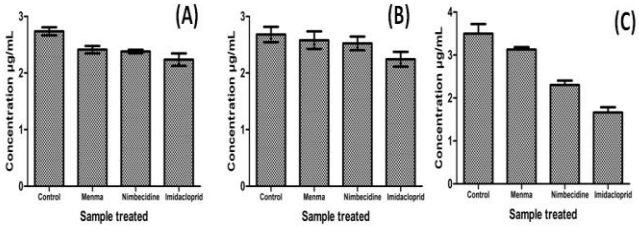

Figure 1: Alterations in the total protein of *O.niloticus* due to the exposures of pesticides in the gill (A), liver (B) and kidney (C)

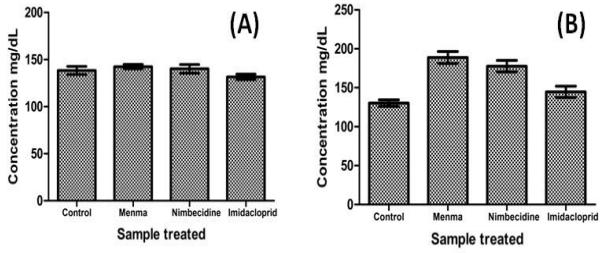

Figure 2: Sub lethal effect of pesticides on the level of serum glucose (A) and cholesterol (B) of *O.niloticus*

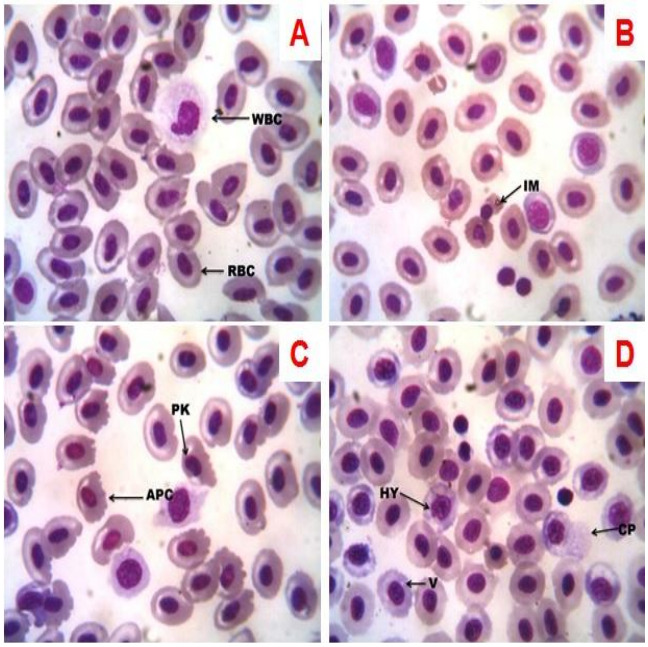

Figure 3: Blood cells of *O. niloticus*. A-Control, B-*Menma* treated: Immature cells (IM), C-Nimbecidine treated: Pyknotic (PK) nucleus, Anisopoikulocytic cells (APCs), D-Imidacloprid treated: Nuclear Hypertrophy (HY), Cytoplasmic Projection (CP), Vacuoles (V). Scale bar = 10 µm. The photomicrographs were captured using a phase contrast microscope (Olympus CKX41 connected with pro5 CCD camera)

4. Discussion

The estimation of biochemical parameters in fish is helpful to understand normal and pathological activities of aquatic organisms as well as toxicological impacts [10]. Proteins play a key role in the structure and function of the cell and occupy a major position in cellular metabolism [11].The depletion of protein fraction could be a consequence of the degradation and possible utilization for metabolic purposes [12]. Decrease in protein level in the present study might have resulted from the high protein hydrolytic activity due to elevation of protease enzyme in the tissues [13]. Reduction in the protein level was also observed in fresh water fish *Ophiocephalus orientalis* by the exposure of Cypermethrin [14].

Increased level of plasma glucose in the present study might be due to the manifestation of stress induced glycolysis by the pesticides [15]. Glucose is the immediate energy source by all body cells and must be maintained at adequate levels in the plasma [16]. Hyperglycemia in fish could be a consequences of the toxic action of pesticides which accelerate gluconeogenesis from the extra hepatic tissue proteins and amino acids [17]. Similar results were also found in *Channa punctatus* when exposed to Imidacloprid [18].The same increased blood glucose activity were also observed in *Cyprinus carpio* exposed with Cypermethrin [19].

Cholesterol is one of the important component of cell membranes and the precursor molecule of all steroid hormones

[20]. Higher blood cholesterol in the present study might have been due to the release of excess reserve energy [21]. It could be a consequence of the impairment of liver along with the inhibition of enzymes which convert cholesterol into bile acids [22] and causing release of additional cholesterol into the blood [23]. The increased cholesterol level in African catfish, *Clarias gariepinus* due to the exposure of lambda Cyclothrin were reported [24]. Previous studies with biopesticide, *Tephrosia purpura* have resulted in increase of cholesterol in fresh water fish *Channa punctatus* [25].

Morphological changes in blood cells are one of the most specific indices of toxic effect on fish [26]. Deformities in shape of blood cells on the exposure of Nimbecidine and Imidacloprid in the present study might be due to the change in membrane lipid composition [27]. Poikilocytosis and anisocytosis of red blood cells may be due to the characteristic feature of megaloblastic, normoblastic and macroblastic anaemia [28], [29]. Similar type of anisocytic RBCs were found in *Clarias batrachus* due to carbaryl effect [30]. The altered forms of blood cells can be assigned due to four main reasons i.e., abnormal erythropoiesis; inadequate hemoglobin formation; damage to the red cells after they leave bone marrow or increased erythropoiesis by bone marrow to compensate for anemic conditions [31].The immature RBCs found in the *Menma* treated fishes were supposed to be due to the acceleration of erythropoiesis in different physiological conditions [32].

5. Acknowledgements

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