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A Comparative study on the Effect of Malathion, Chlorpyriphos and Neem on lipid peroxidation in fresh water fish *Anabas testudineus*

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Abstract

A comparative study on the impact of sub lethal acute toxicity of pesticides Malathion, Chlorpyriphos and Neem on lipid peroxidation in fresh water fish *Anabas testudineus* was done.The dosage selected was LD_{10}. Lipid peroxide content in liver, kidney and gills of each group was estimated. The study was conducted for 24 and 48 hours. Increased levels of lipid peroxide content were observed during the exposure of Malathion and chlorpyriphos. While neem resulted least enhancement of lipid peroxide content. Observations on the levels of lipid peroxide content showed that chlorpyriphos has more destructive effect than malathion which is indicated by the elevated levels of lipid peroxide content. Levels of lipid peroxide content can be taken as an index for monitoring the extent of pesticide contamination in the aquatic ecosystem. The study illustrated that chlorpyriphos and Malathion are harmful to aquatic organisms like fishes at sub lethal concentrations and the application of insecticides in agriculture fields close to water bodies adversely affect the aquatic fauna.

Key Words : Lipid Peroxidation, Malathion, Chlorpyriphos, Neem

Introduction

Fisheries and aquatic resources are the most valuable natural resources. Growing human populations and human activities adversely affected the aquatic life and water quality. Pesticides are a major group of toxic compounds that have a profound effect on aquatic life and water quality. Pesticides are chemicals used to eliminate or control a variety of agricultural pests that can damage crops and livestock (Al-Saleh .A,1994).These compounds are selectively formulated for specific toxic properties. Increasing demand for food for millions of people and for controlling the vectors of human diseases are the two pressing problems directly related to pest control (Daly H, Doyen J.T, and Purcell A.H,1998).. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, because they are sprayed or spread across entire agricultural fields. (George Tyler Miller 2004). Pesticide surface runoff into rivers and streams can be highly lethal to aquatic life, sometimes killing all the fish in a particular stream.Pesticides can accumulate in bodies of water to levels that kill off zooplankton, the main source of food for young fish (Helfrich, LA, Weigmann et al 1996).

Malathion, chlorpyrifos, and neem are the pesticides used in this study.Malathion is an insecticide in the chemical family of organophosphates . Bioactivation of malathion create the active metabolite malaoxon through oxidative sulfuration.Malaoxon is considered to be 22 times more toxic than the parent malathion .(Tomlin, C.D.S 1997).Chlorpyrifos is also an organophosphate very highly toxic to aquatic invertebrates, freshwater fishes, and other estuarine and marine organisms.There is potential for chlorpyrifos to bioaccumulate in the tissues of aquatic species. (Muller and Franz, 2000). The pesticidal characteristics of neem which is a biopesticide is largely attributable to Azadirachtin found in the neem extracts which is a growth regulator and as well as a powerful feeding and ovipositional deterrent.(Schmutterer.H,1998)

Lipid peroxidation involves the formation and propagation of lipid radicals, the uptake of oxygen, a rearrangement of the double bonds in unsaturated lipids and the eventual destruction of membrane lipids, with the production of a variety of breakdown products, including alcohols, ketones, alkanes, aldehydes and ethers (Marnett L J. 1999). The mechanism of biological damage and the toxicity of these reactive species on biological systems are currently explained by the sequential stages of reversible oxidative stress and irreversible oxidative damage. Oxidative stress is understood as an imbalance situation with increased oxidants or decreased antioxidants .

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Materials and Methods

Healthy specimens of Anabas testudineus were collected from Vellayani lake which is a fresh water lake located 12 Km south west of Thiruvananthapuram city. Almost same sized fishes with a length of 13-15 cm and body weight of 19-20 g were used for the experiment. They were maintained in the laboratory in large glass aquaria containing weathered, well aerated tap water for two weeks. The fishes were treated with potassium permanganate solution (0.5% w/v) to get rid of infectious organisms. They were fed with standard food pellets ad libitum. Pesticides used for the study were Malathion , Chlorpyriphos and Neem(Tradename is Nymbosol) . All were obtained from local markets in Thiruvananthapuram.

Experimental Design

The fishes were divided into four groups of six each and kept in four aquarium tanks Of 6L (120 cm x 45 cm x 80 cm) containing dechlorinated water.

Group I-Control

Group II- exposed to malathion.

Then group III - exposed to chlorpyrifos and

group IV-Exposed to neem.

The dosage selected was LD_{10}. Accordingly the dosage of Malathion was 0.0083ppm, Chlorpyrifos was 0.005ppm and Neem was 500ppm per Kg body weight of fish.The dosage was determined through pilot tests. Each group was used for the quantitative estimation of lipid peroxide content in liver, kidney and gills .Effect of pesticides on lipid peroxidation for24 and 48 hours were studied. At the end of the experimental period , fishes were anaesthetized by chloroform and were dissected. Tissues (liver, kidney, gills) were taken from each group and the weighed tissues were homogenized in 0.89% KCl by mortar and pestle. Then the homogenates were centrifuged at 3000 rpm for 10 minutes. Supernatants were used for the estimation.

Estimation of Lipid Peroxid Content

Lipid peroxide content was estimated by thiobarbituric acid method .(W. G. Niehaus JR.,B. Samuelsson 1968). The system contained TBA-TCA-HCl reagent. The Malondialdehyde(MDA) formed from the breakdown of poly unsaturated fatty acids serves as a convenient index for determining the extent of peroxidation reaction. MDA has been identified as the product of lipid peroxides that reacts with TBA to give a red colour absorbing at 532nm.

Results and Discussion

Exposure of pesticides resulted increased levels of lipid peroxide content (LPC)in liver, kidney and gills both during 24 and 48 hours of exposure. The results are graphically represented in Figure 1 and 2. Chlorpyrifos and Malathion induced higher levels of lipid peroxide content than control. Neem resulted least induction of LPC than the other two pesticides.Among the three pesticides ,lipid peroxide content was higher in chlorpyriphos exposed groups. The results indicate that lipid peroxidation was higher in liver followed by gills. The effect of pesticides on lipid peroxidation during 48 hour exposure was higher than that of 24 hour .

In the present study it was observed that Malathion/ Chlorpyriphos exposed fishes showed increased physical activity including restlessness and decreased rate of breathing. But in fishes exposed to neem extract, the physical activity and breathing rate remained more or less normal. Many studies indicated that organophosphate pesticides inhibit the activity of the enzyme acetyl cholinesterase (Andrea Slaninova,Miriam.S .mutna etal -2009). Acetylcholine that bind with sub synaptic membrane will not easily get degraded due to the decreased activity of acetylcholine esterase and will result cholinergic hyperactivity.

The oxidative stress is an inescapable component of aerobic life. In the healthy aerobic organism, a balance between the reactive oxygen species (ROS) production and the system to protect cells from ROS exists. ROS production results in defects that may cause cell damages or organism's death. This imbalance is referred to as oxidative stress.

From the results, it can be seen that chlorpyriphos is more potentially toxic than Malathion and neem is least toxic. Although the main mode of action of organophosphates is the AChE inhibition, the increase in lipid peroxidation and changes in ATPase activity were observed in context to the toxicity of these pesticides. The long-time administration of organophosphates caused a gradual exhaustion of the enzymes Superoxide dismutase(SOD), Glutathione reductase (GR),Glutathione peroxidase(GPX) and Glutathione- Sransferase (GST) which ultimately leads to higher lipid peroxidation (Fujioka K, Casida JE-2007)

A large number of toxic by-products are formed during lipid peroxidation (LP). These have effects at a site away from area of their generation. Hence they behave as toxic 'second messengers'. Membrane lipids are particularly susceptible to LP. Since membranes form the basis of many cellular organelles like mitochondria, plasma membranes, endoplasmic reticulum, lysosomes, peroxisomes etc , the damage caused by LP is highly detrimental to the functioning of the cell and its survival. Presence of polyunsaturated fatty acids (PUFAs) in the phospholipids of the bilayer of biological membranes is the basis of their critical feature of fluidity. Since lipid peroxides attacks the components that impart these properties, it affects the biophysical properties of membranes. LP decreases the membrane fluidity, changes the phase properties of the membranes and decreases electrical resistance. Also, cross-linking of membrane components restricts mobility of membrane proteins. Peroxidative attack on PUFAs of a biological membrane will compromise one of its most important functions: its ability to act as barrier. Hence, LP causes lysosomes become fragile or simply 'leaky'and may leads to the destruction of the cell itself. (Jirasak Wong-ekkabut,*† Zhitao Xu,* Wannapong Triampo, et al 2007).

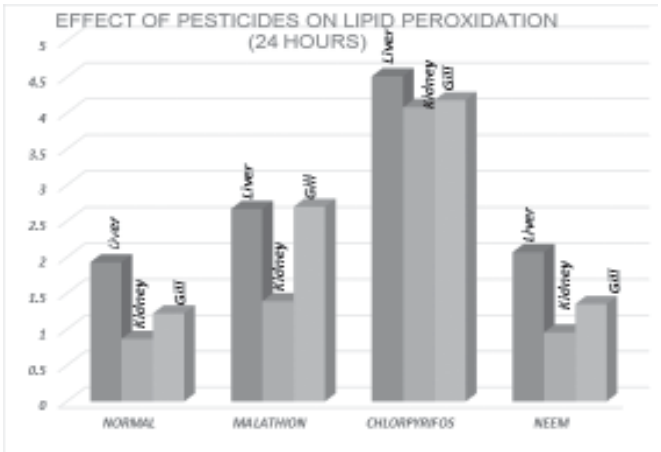
Mosquito fish (Gambusia affinis) exhibits the inhibition in activities of catalase, SOD and reduced glutathione and increased LPO and Malon di aldehyde(MDA) level after the exposure of chlorpyrifos

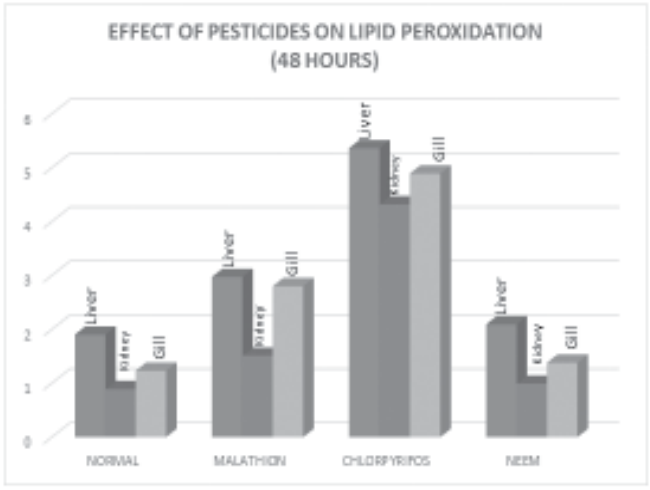
for 96 h. It suggest that the ROS-induced damage plays a main role in the toxic effect of chlorpyriphos. The consistent decrease in the antioxidant enzymes was due to the excessive generation of free radicals generated by chlorpyrifos. The normal levels of antioxidants could not quench the excess of free radicals and thereby increased lipid peroxidation. (Hai DQ, Varga IS et al,1995).In Vitro studies in rat tissues showed higher lipid peroxide level in chlorpyriphos exposure.(GultekÝn, F.; Ozturk, M.; Akdogan, M.,2000) Effect of Chlorpyrifos and Lead acetate on lipid peroxidation and antioxidant enzyme activity showed higher levels lipid peroxide content in different rat tissues (Nisar Ahmad Nisar, Mudasir Sultana etal,2017)

Malathion and chlorpyrifos exposed groups exhibited highest level of MDA content in liver followed by Kidneys. This might be due to the elevated oxidation of molecular oxygen (O_2) to produce superoxide radicals, indicating the importance of Liver and kidney in the detoxification process. The activities of the endogenous enzymes to remove the continuously generated free radicals initially increase due to induction but later enzyme depletion occurs, resulting in higher lipidperoxide content and oxidative cell damage (V. Seth. A. Bhattacharya.,1999) As mentioned earlier metabolism of pesticides results the products of lipid peroxidation as well as the free radicals formed could cause irreversible damage to the cell structure. Sometimes it may result the cell death.It was reported that Malathion caused oxidative stress in different tissues of mice for a maximum exposure period of 30 days.The degree of lipid per oxidation increased in ascending order.The findings indicate that the continuous and prolonged exposure of Malathion in sub lethal dose resulted in the induction of lipid peroxidation(Varsha W. Wankhade , 2012 **)**

Conclusion

The knowledge in oxidative stress in fishes has great importance in environmental and aquatic toxicology. Oxidative stress is evoked by many chemicals including some pesticides and the oxidant factors' action in fishes can be used to assess pollution of a specific area.Present study illustrates that chlorpyriphos and Malathion induce lipid peroxidation in fishes even at low concentration and short period of exposure. While neem is a safer insecticide with least induction of lipid peroxidation.





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