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Sub-Lethal Effect of Monocrotophos Pesticide on Risk Appraisal and Environmental Chemical Regulation of Muscle and Liver Fatty Acid of *Channa Striatus*

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Abstract

The aim of this study was to investigate the sub-lethal toxicity of a monocrotophos organophosphate insecticide effect of muscle and liver tissues fatty acid profile of *Channa striatus*. To evaluate the toxic effects of monocrotophos organophosphate insecticide from agricultural application, monocrotophos organophosphate insecticide contained sub-lethal concentration of 96 hours of monocrotophos organophosphate insecticide exposure was 0.98 ppm and 1 ppm, duration of 15 day and 30 days for mural, *C. striatus*. The sub-lethal concentration used for monocrotophos organophosphate insecticide was disturbed biochemical parameters and produced liver and muscle tissue fatty acids damages. In muscles and liver tissue estimation monocrotophos insecticide effects significantly (P>0.05) decreased in crude protein and carbohydrate. Free fatty acid profile of *C. striatus* of saturated (SFAs), mono unsaturated (MUFAs) and poly unsaturated fatty acids (PUFAs) were increased in muscle tissue 0.98 ppm 30 days and liver tissue 1 ppm 15 day while compared to control. Estimation of muscle and liver tissue saturated fatty acids (SFAs) as capric acid methyl ester, caprylic acid methyl ester, undecanoic acid methyl ester and tricosanoic acid methyl ester were decreased level of monocrotophos 50% insecticide treated groups when compared to control. Simultaneously, palmitic acid methyl ester was increased in muscle and liver tissue of 1ppm 15 days and 1ppm 30days, while compared to control, respectively. In conclusion, modify observed among the chemical composition, fatty acid profile and free fatty acid investigation of muscle and liver in toxic effect of monocrotophos 50% insecticide of gas chromatograph evaluation.

Keywords
Sub-lethal concentration, GC-MS, *Channa striatus,* fatty acid.

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INTRODUCTION

Adversely human activities are directly or indirectly affecting the environment. Due to development activities such as construction, transportation and manufacturing not only deplete the nature resources but also produce large amount of wastes that leads to pollution of air, water and soil. Today environmental pollution has become not only a national but also an international problem (Tamizhazhagan & Pugazhendy, 2015). Water pollution is usually caused by various human sources, typically (point and non-point) industrial facilities and agrochemicals especially in aquatic ecosystem, has grown up to be a serious environmental problem nowadays.

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). Adversely human activities are directly or indirectly affect the environment (Jayakumar et al., 2018). Pesticide is widely used in modern agriculture to aid in the production of high-quality food. However, some pesticides have the potential to cause serious health and environment damage. Repeated exposure to sub-lethal doses of some pesticides can cause physiological and behavioral changes in fish that reduce populations, such as abandonment of nests and broods, decreased immunity to disease and increased failure to avoid predators (Veeraiah, 2012). The inorganic pesticides include borates fluorides and mercurial Natural organic compounds including pyrethrum, rotenone and nicotine6 and the synthetic organic compounds includes chlorinated hydrocarbon, organophosphates and cremates (Tamizhazhagan et al., 2017).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. These wastes mostly contain various types of pollutants such as heavy metals, radioactive elements, pesticides, herbicides and corrosive substances like acids and bases (Mhadhbi et al., 2012). 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 (Kasinathan et al., 2018). The organophosphorous compounds are widely used because of their rapid biodegradability and non-persistent nature. Recently studies have proved that extremely low quantities of pesticides which enter the aquatic environment can affect productivity of organisms to kill eggs and larvae (Padmapriya et al., 2017).

Global aquatic ecosystems fall under two broad classes defined by salinity – freshwater ecosystem and the saltwater ecosystem. Freshwater ecosystems are inland waters that have low concentrations of salt. The salt-water ecosystem has a high concentration of salt content (averaging about 3.5%). The study of freshwater habitats is known as limnology phytoplankton taxonomic composition and in order lead to the eutrophication and algal blooms(Béthoux et al., 2002; Piehler et al., 2004). Residues of toxic chemical found in water, sediment, fish and other aquatic biota can pose a risk to organisms to predators and human being (Jayalakshmi et al., 2017). This may lead acetate to the destruction of beneficial species either indirectly through breaking the biological food chain or directly by affecting the aquatic forms of life(Pichaimani et al., 2017a). A few other notable antioxidant enzymes that are rendered inactive by lead acetate include superoxide dismutase (SOD) and catalase (CAT). Decrease in SOD concentration reduces the disposal of superoxide radical, whereas reduction in CAT impairs scavenging of superoxide radical (O2–) (Pichaimani et al., 2017b). AChE is an important enzyme that can be measured environmental bio-indicator in the animal body. Cholinesterase is involved in the signal transmission at neuromuscular junctions and is also intensely expressed in the organism nervous system. Usha et al., (2017a) the random use of different pesticides often causes lot of damage on non-target organism. Fish immune system, important for defense against a variety of harmful pathogens is very sensitive to homeostatic adjustments via endocrine regulation and is influenced by the biochemical profile of the nervous system (Usha et al., 2017b). Paradoxically, they are highly vulnerable to toxic chemicals because firstly, their large surface area facilitates greater toxicant interaction and absorption and secondly, their detoxification system is not as robust as that of liver (Tamizhazhagan et al., 2016). 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). Chemical pesticides have contributed greatly to the increase of yields in agriculture by controlling pest and disease and also towards clacking the insect-borne disease (malaria, dengue, encephalitis, filariasis, etc.) in human health sector (Rahman & Siddiqui, 2006). Now day's farmers are using an assortment of pesticide and insecticide monocrotophos in their grassland of cultivation devious the insect pest. 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).

MATERIALS AND METHODS

Fish collection and laboratory conditions

The freshwater healthy fish *Channa striatus* (Figure 1) of the weight (26.34 ± 0.79g) and length (17 to 20cm) were selected for the experiment and were collected from ponds in around Lowe anaicut, Anaikkarai. 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 tanks containing dechlorinated tap water (Temperature 26°C).

Figure 1. *Channa striatus*

Experimental design

The experimental group was vulnerable to a sublethal concentration of the insecticide (0.54ppmL-1) during 15 and 30 days. Toxicity tests carried out in accordance with standard methods (APHA, 1800). Stock solution of arsenic with a concentration of 1g per litre (equivalent to 1 ppt) was prepared in distilled water and different, dilutions were prepared by adding the required amount of distilled water. Based on the progressive bisection of intervals on a logarithmic scale, log concentrations were fixed after conducting the range finding test. The fishes were starved for 24 hours prior to their use in experiments as recommended by storage, to avoid any interference in the toxicity of pesticides by excretory products (Figure 2).

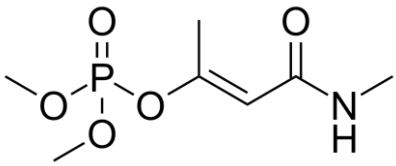

Figure 2. Monocrotophos organophosphate insecticide chemical structure

Sub-lethal concentration

Based on acute toxicity test (96h LC50) sub-lethal concentrations (1ppm and 2ppm 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.

Moisture content

The moisture content of the fish species was identified using the air oven drying method using a known weight of the fillet at 105°C until a constant weight was obtained (Isaac et al., 1994).

Total Protein

The total protein content of the insecticide exposed tissue samples were estimated according to the modified universal method (Lowry et al., 1951).

Total carbohydrate

The total carbohydrate content estimated by the method of (Hedge et al., 1962).

Total lipids

Total lipids in muscle and liver tissues were estimated by the method of (Folch et al., 1957). The pink colour developed was read at 530nm. Blank was established by adding 5ml of vanillin reagent to 1ml of distilled water. Total lipid content was expressed as mg/g tissue.

Muscle and liver sample preparation for fatty acid analysis

Lipid extraction followed the (Bligh & Dyer, 1959). Methyl esters were prepared by transmethylation using 2M KOH in methanol and n-heptane according to the method as described by Ichihara (1996). Extract lipids (10mg) were dissolved in 2 ml heptane followed 4 ml of 2 M methanolic KOH. The tube was vortexed for 2 minutes at room temperature. After centrifugation at 4000 rpm for 10 minutes, the heptane layer was taken for GC analyses. Cap tubes with rubber caps, vortex, and let stand at room temperature for 20 minutes. Dissolve samples in 50 µl iso-octane and transfer to label sample vial with 250 µl glass inserts. Cap and place samples in the GC-MS sample tray and begin analysis.

Fatty acids analysis through gas chromatography (GC)
The profile of fatty acids was completed following gas chromatographic (GC) method (Foley, Nichols, & Myers, 1993). Two oils of liver and muscle tissue were injected and analyzed utilizing Chemito 8610 Gas chromatography, with BPX70 capillary column and flame ionization detector. Both standard mixture and each of the fatty acid methyl esters of the analyzed samples were chromatographically separated under the same conditions, using the same temperature program (oven initial temperature 140ºC to final temperature 240ºC, heating rate 4ºC/min.), split rate 100:1. Nitrogen was invoked as carrier gas. The chromatogram was used only for calculation. Standard fatty acids were analyzed simultaneously. Based on the retention time and peak, area of the standard fatty acids, each fatty acid in the unknown sample was identified. The calibration of the signals was made by taking into consideration the concentration of each component of the standard mixture, correlated with the detector's response. Fatty acids were identified by comparing the retention time of FAME with a standard 37 component FAME mixtures (Supelco, USA). Two replicate GC analyses were performed.

Statistical Analysis

All the dates were subject to one-way ANOVA using statistical software of SPSS version 16.0. Duncan's Multiple Range test was used to establish the difference among treatment means at 5% level of significance.

RESULTS

Aquatic toxicology was the effect of environmental contamination on aquatic animals, such as the effect of pollution on the health fish or other aquatic organisms. The muscles composition of moisture, protein and carbohydrate were decreased in the insecticide monocrotophos concentration of 0.98 ppm of 30 days (80.00±0.8195, 21±0.95, 39.18±0.96 and 56.59±0.81) when compared to another concentration of 1ppm of 30 days (80.20±0.88, 109.72±0.81, 42.16±0.82 and 65.95±0.44) and control (81.60±0.81, 118.20±0.96, 51.31±0.93 and 74.65±0.81) respectively. Simultaneously, lipid composition was reduced in 1ppm of 30 days when comported to other treated groups and control, respectively (Table 1). The liver sample composition of moisture, portion, lipid and carbohydrate were decreased in the insecticide of monocrotophos concentration of 0.98 ppm of 30 days (81.30±0.81, 89.95±1.02, 112.54±0.87, 46.21±1.33, and 9.12 ± 0.73) while compared to another concentration of 1ppm of 30 days (81.14±0.71, 96.75±0.76, 98. 41.15±1.04 and 6.14±0.71) and control (82.10±0.82, 132.46±0.75, 64.86±1.14 and 11.08±0.75) respectively (Table2).

Table 1: Chemical composition of muscle and liver of *C. striatus* exposed to sub-lethal concentration of Monocrotophos

Treatment		Muscle Moisture	Protein (mg/gm tissue)	Carbohydrates (mg/gm tissue)	Lipid (mg/gm tissue)
Control		81.60±0.81^{a}	118.20±0.96^{a}	51.31±0.93^{a}	74.65±0.81^{a}
0.98ppm	15 days	80.20±0.88^{ab}	109.72±0.81^{b}	42.16±0.82^{b}	65.95±0.44^{b}
	30 days	80.00±0.81^{ab}	95.21±0.95^{c}	39.18±0.96^{c}	56.59±0.81^{c}
1ppm	15 days	79.30±1.14^{ab}	82.35±0.81^{d}	41.36±1.02^{b}	67.73±0.93^{b}
	30 days	78.40±0.69^{b}	71.29±1.21^{e}	29.87±0.81^{c}	58.91±0.81^{c}
Liver					
	Days	82.10±0.82	132.46±0.75^{a}	64.86±1.14^{a}	11.08±0.75^{a}
0.98ppm	15 days	81.30±0.81	112.54±0.87^{b}	46.21±1.33^{c}	9.12±0.73^{b}
	30 days	81.14±0.71	96.75±0.76^{d}	41.15±1.04^{d}	6.14±0.71^{c}
1ppm	15 days	81.50±0.93	102.34±0.86^{c}	50.36±0.90^{b}	5.28±0.69^{c}
	30 days	81.00±0.81	88.95±1.31^{e}	39.95±1.33^{d}	4.54±0.65^{d}

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).

Table 2: Free fatty acids profile in muscle and liver tissue of *C. striatus* exposed to sub-lethal concentration of Monocrotophos.

Treatment		Muscle ∑SFA	∑MUFA	∑PUFA
Control		1.371±0.156^{c}	1.612±0.258^{b}	0.41±0.129^{cd}
0.98ppm	15 days	7.401±1.044^{a}	1.383±0.258^{c}	2.242±0.299^{b}
	30 days	4.052±0.843^{b}	5.504±0.745^{a}	4.182±0.596^{a}
1ppm	15 days	1.15±0.218^{c}	0.42±0.042^{c}	0.766±0.015^{e}
	30 days	2.372±0.298^{ab}	0.681±0.062^{c}	1.029±0.149^{c}
Liver				
0.98ppm	15 days	2.704±0.421^{d}	0.844±0.083^{b}	1.698±0.363^{b}
	30 days	2.303±0.856^{e}	0.621±0.093^{b}	0.596±0.137^{b}
1ppm	15 days	10.013±0.143^{a}	12.549±1.490^{a}	10.08±2.115^{a}
	30 days	6.614±0.336^{b}	0.96±0.723^{b}	1.443±0.257^{b}

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).

The results of average deprived of fatty acid and free fatty acid levels in muscle of *C.striatus*, at different periods of exposures are presented in table 3 & 4 and figure 1. Values of muscle tissue saturated fatty acids (SFAs) of Caproic acid methyl ester, Caprylic acid methyl ester, Capric acid methyl ester, Myristic acid methyl ester and Heptadecanoic acid methyl ester was reduced in different concentration of treated group, beside Lignoceric acid methyl ester were extant in 1ppm of 30day of monocrotophos insecticide treated groups only, not distinguished, while compared to other concentrated of monocrotophos insecticide groups as well as Palmitic acid methyl ester was increased then compared to control, respectively (Table 1). Mono Unsaturated FAs (MUFAs) of Myristoleic acid methyl ester, Cis-10-Pentadecenoic acid methyl ester, Palmitoleic acid methyl ester, Cis-10-Heptadecenoic acid methyl ester, Oleic acid methyl ester, Nervonic acid methyl ester, Oleic acid methyl ester and Nervonic acid methyl ester was weaken in different concentration of monocrotophos insecticide treated groups when compared to control, respectively (Table 2). Saturated fatty acids in muscle tissue were inferior of increased in treated group of 1ppm of 15 days (7.401±1.044) where compared to another treatment groups of monocrotophos insecticide (1.383±0.258, 2.242±0.299) and control (1.371±0.156), respectively (Table 1). Monounsaturated fatty acids were of inferior quality increased in monocrotophos 1ppm of 30days (1.383±0.258) while compared to another treatment groups (5.504±0.745, 0.42±0.042, 0.681±0.062) and control (1.612±0.258) in muscle tissue, respectively (Table 2).

DISCUSSION

Moisture, crude protein and carbohydrate were decreased in monocrotophose treated groups. Carbohydrates form one of the major sources of energy precursor under any stress condition. Carbohydrate levels clearly indicate its rapid utilization to meet the enhanced energy demands for pesticides treated individuals through glycolysis or hexose monophosphate pathway. Total carbohydrate content decreased during the exposure to monocrotophos in the air breathing fish Anabas scandens maximum decrease in the brain tissues (Khan et al., 2016). Decreased carbohydrate level has been noted in the liver and muscle of Heteropneustes fossils exposed to herbicide (Sharma & Agarwal, 2004). Several investigators have reported a number of changes in biochemical parameters of aquatic organisms due to pesticide exposure (Grewal et al., 2009; Remia et al., 2008). The pesticides are also known to inhibit energy synthesis by suppressing aerobic oxidation of protein leading to energy crisis in animals (Kohli et al., 1977). Proteins are complex substance with high molecular compound weight from not only the structural framework, but also gears and levers of the operating mechanism in the living wage body. The protein content of the muscle and liver of Catla catla was decreased with the low concentration of pesticide in Monocrotophos. Even with the same concentration longer exposure resulted in decreased amount of protein content which indicates that the tissue protein endures proteolysis.

Fish proteins are well balanced with essential amino acids and are comparable to other proteins of animals origin (Tont et al., 1977) , further fish contain lipids especially omega fatty acids and free fatty acid from the human nutritious point of view. Toxicity data for a variety of pesticides such as organophosphate,

organochlorine, carbamide and pyrethroid pesticides have been reported for number of fish species by various author's (Gurusamy & Ramadoss, 2000; Kavanaugh et al., 2009; Muthukumaravel et al., 2013). The natural physiological functioning of an organisms gets distributed on exposure to toxicants, stress, it induces effect first at cellular or even at molecular level, but ultimately cause physiological, pathological and biochemical alteration (Venkata Rathnamma & Nagaraju, 2013). Fatty acids are kept in the cytosol as triglycerides. Fatty acids are released from triglycerides by the action of lipases. To begin the oxidation process, the fatty acid is triggered by converting the carboxylic acid to thioester to coenzyme A, generating acyl- CoA. The acyl-CoA is transported into the mitochondrial matrix where oxidation occurs (Venkata Rathnamma & Nagaraju, 2013). Liver is a recognized target organ for metabolism exogenous toxins and synthesizing fatty acids. It's many physiological and molecular similarities in exogenous toxin metabolism and adaptive responses between zebra fishes and mammals (Spitsbergen & Kent, 2003), it is feasible to apply zebra fish as an in vivo system to model human diseases and toxicity. In this study, we are aimed to quantify biological responses and ensuing risks of developing certain diseases based on quantitative analysis of the fatty acid composition of hepatic lipids. It was an interesting note that Lignoceric acid which was present in pesticide treated group, was absent in control. In the present study, reduction in unsaturated fatty acids in liver and muscle could be explained by their utilization for energy purposes.

Similarly to the present study, Morales et al., (1999) reported that the reduced of olic acid, arachidonic acid and n-3 HUFA (highly unsaturated fatty acid) in liver of Sparus aurata juveniles stocked at high stocking density and was attributed to meet increased energy demand. There were much higher changes in the level of C16:0 and C18:0 fatty acids in hepatic lipids of C. striatus although they have similar changes in polyunsaturated long chain fatty acids in response to monocrotophos insecticide exposure. This result implies that sex hormones may play critical roles in fatty acid metabolism. In fact, lots of experimental results have already demonstrated the important roles of hormones in lipid mobilization, which is another important pathway directly impacting the quantity of fatty acids.

CONCLUSION

In this investigation exposed that sub-lethal effect of monocrotophos on chemical composition and fatty acid profile of *C. striatus.* Environmental monocrotophos exposure can cause changes in the fatty acid composition of *C. striatus* muscle and liver tissue. With increased concentration of monocrotophos exposure, the content of long chain saturated fatty acids (SAFAs) C16:0 and C18:0 as well as long chain monounsaturated fatty acids (MUFAs) C18:1n9 in hepatic tissue consistently increased while long chain polyunsaturated fatty acids (PUFAs) C20:4n6, C20:3n3, and C22:6n3 in hepatic lipids decreased. It has been revealed that *C. striatus* had much higher changes in the level of saturated fatty acids including C16:0 and C18:0 in a monocrotophos concentration dependent.

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