



Sub-lethal effect of methomyl-based pesticide on chemical compositions and fatty acid profiles of fresh water fish *Channa striatus*

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Abstract

The aim of this study was to investigate the sub-lethal toxicity of a methomyl-based insecticide effect of muscle and liver fatty acid of *Channa Striatus*. Methomyl-based insecticides were treated sub-lethal concentration of 96 hours of methomyl exposure was 1 ppm and 2ppm, duration of 15 day and 30 days for murrel, *Channa striatus*. The liver and muscle tissue fatty acids were analyzed. In muscles and liver tissue estimation methomyl 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 1ppm 30 days and liver tissue 2ppm 15 day while compared to control. Estimation of muscle and liver tissue saturated fatty acids (SFAs) as caprylic acid methyl ester, capric acid methyl ester, undecanoic acid methyl ester and tricosanoic acid methyl ester were decreased level of methomyl 40% insecticide treated groups when compared to control. Simultaneously, palmitic acid methyl ester was increased in muscle and liver tissue of 1ppm 15 days and 2ppm 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 methomyl 40% insecticide of gas chromatograph evaluation.

Keywords: sub-lethal concentration; gas chromatograph, palmitic acid methyl ester; *Channa striatus*

Introduction

Methomyl is a highly toxic carbamate insecticide. The most popular of these pesticides for residential uses is carbaryl and propoxur. 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 (Trachantong *et al.*, 2017). 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 India. Organophosphate pesticides also inhibit this enzyme, although irreversibly and cause a more severe form of cholinergic poisoning (Tamimi *et al.*, 2008) [43]. Pesticides are generally used in contemporary agriculture to aid in the manufacture of high quality food (Tamizhazhagan *et al.*, 2017) [20, 23, 31, 44, 51]. Their pesticide compounds were leads to some effect in animals. Fish food possesses high nutritional quality and particularly recommended human dietary component.

The global toxic effects of environmental chemicals have been achieved by using genomic and proteomic tools in the past years (Sanchez *et al.*, 2011) [36]. Fish meat possesses high nutritional quality and is, therefore, a particularly recommended human dietary component. Information concerning the chemical and fatty acid composition of freshwater fishes is valuable to nutritionists who are interested in finding sources of low-fat, high protein foods, with desirable fatty acid compositions and acceptable amount of total cholesterol. Fish meat contains biologically active

protein which is characterized by a very favourable composition of amino acids, a high omega-3 polyunsaturated fatty acid content such as Eicosapentaenoic acid (20:5 n-3, EPA) and Docosahexaenoic (22:6 n-3, DHA) and fat-soluble vitamins as well as it represents a good source of micro- and macroelements. The shortage of α -Linolenic acid (18:3 n-3, ALA) is responsible for neurological disorders and poor growth (D.K Cundiff., *et al* 2007) [11]. Fish food uses a generous quantity of diet to avoid excessive consumption of saturated fatty acids and as a means for the human to obtain adequate protein in the diet without taking in excessive fat, which might result in their becoming overweight.

Nowadays, the use of pesticide is widespread on cultivating crops, rangelands, forests, and wetlands and this undoubtedly exposes many wildlife species to chemical hazards. Various pesticides need to be resistant to environmental degradation so that they persist in treated areas and thus their effectiveness is enhanced (Ramasamy *et al*, 2007; Jayalakshmi *et al.*, 2017) [21, 23]. The snakehead (*Channa striatus*) fish provides a good source of albumin for people who have low albumin serum in post-operative condition. In rural areas, the snakefish is traditionally administered to eat who are just after having circumcised to accelerate the healing process. Lipids constitute one of the most important components of fish, muscle providing energy reserves and components of cell bio-membranes. The lipid is extracted from the flesh for the analysis of lipid composition. Fish flesh is an important part of the fish, which people eat and are considered the important source of protein. Fish lipids are dominated by saturated fatty acids like palmitic (C16:0) and myristic (C14:0) acids and stearic acid, whereas the monosaturated fatty acids (MUFA)

are oleic and palmitoleic acids (Kolakowska., 2002) [24] and while Polyunsaturated fatty acids (PUFA) are Eicosapentaenoic acid (EPA) and Docosahexaenoic acid, which may reduce the risk of coronary heart diseases (Bhaskar, 2006 and Dhanapal, 2011) [5, 12].

Fatty acids play a vital role in membrane architecture by maintaining appropriate of a biological membrane (Blem, 1992) [9]. Among straight-chain fatty acids, the simplest is referred saturated fatty acids. They have no unsaturated linkages and cannot be modified by hydrogenation or halogenation. The simplest FAs are named saturated fatty acids (SFAs), which have no unsaturated linkages. They are highly flexible because they have full rotation around each carbon bond. When double bonds are present, FAs are defined as unsaturated: monounsaturated (MUFAs) if only one double bond is present, and polyenoic (or polyunsaturated fatty acids, PUFAs) if they contain two or more double bonds (Boyer, 2000) [8]. In recent physiological work, used only for fatty acids with three up to six double bonds as those found in fish oil. Some uncommon polyunsaturated fatty acids have two adjacent double bonds separated by more than one methylene group, they are named polymethylene-interrupted fatty acids. Except for fatty acyl-CoA, have based classification of fatty acids first on the type of carbon chain either straight (or normal) or branched or containing a carbon ring. In each category, subdivisions are created according to the functional groups substituted on the carbon chain (Bierman 2000) [6].

Hence, the present investigation is taken up to analyze the sub-lethal concentrations of toxicity of Methomyl on the fat reserves of the liver and muscle determines the proximate, fatty acid composition of the freshwater snakehead fish (*Channa striatus*).

Materials and Methods

Fish collection and laboratory conditions

The freshwater healthy fish *Channa striatus* of the weight (22.34±0.79g) and length (17 to 20cm) 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). 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 faeces and food remnants.

Chemical and Experimental design

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

The experimental group was vulnerable to a sub lethal concentration of the insecticide (0.54ppm L-1) during 15 and 30 days. Toxicity tests carried out in accordance with in standard methods (APHA, 1992) [2]. A stock solution of methomyl with a concentration of 1g per liter (equivalent to 1 ppm) 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. After the addition of the toxicant into the test tank with 10 liters of water having twenty fish, mortality was recorded after 24, 48, 72 and 96 hours. Five replicates were maintained simultaneously.

Sub-lethal concentration

Based on acute toxicity test (96h LC₅₀) sub-lethal concentrations (1ppm and 2ppm of 15 & 30 days) were derived from methomyl which served as the experimental concentration of the methomyl 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

After Morphometric measurements, each fish was dissected to collect diverse organs and tissues. These fish were then muscle and liver transferred in to mark sterilized polythene bags and stored in a freezer at 20°C until further analysis.

Chemical composition

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 (Arlington, 1994) [4].

Total Protein

The total protein content of the insecticide exposed tissue samples were estimated according to the modified universal method (Lowry *et al*, 1951) [25].

Total carbohydrate

The total carbohydrate content estimated by was estimated by the method of Hedge and Hofreiter, (1962) [18].

Total lipids

Total lipids in muscle and liver tissues were estimated by the method of Folch *et al.*, (1957) [15]. One gram of liver and muscle tissue was collected and mixed thoroughly in two ml of chloroform and methanol mixture (2:1 v/v). The reaction mixture, 1ml of 0.9% sodium chloride solution was added and allowed to stand for a few hours. The lower phase was separated and chloroform was dried under vacuum desiccators. The precipitate was dissolved in 1ml of sulphuric acid and volleyed. This mixture was filled into boiling water bath for 10 minutes. After boiling, the mixture was cooled to room temperature, then 0.5ml of the acid digest was taken and 5ml of vanillin reagent was added, mixed well and incubated for 30 minutes. The pink color developed was read at 530 nm. 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 and Dyer method (1959).

Methyl esters were prepared by transmethylation using 2M KOH in methanol and n-heptane according to the method as described by Ichihara (1996) [19]. 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. Used 16 mm 125 mm glass tubes, prepare the sample as stated above based on sample type. 3 extra samples with only internal standard and dPBS are also prepared. 1mL iso-octane was added and the sample was vortexed and centrifuged at 3000g for 1 minute to separate layers. The top layer was removed and transferred to a 10mm 75mm glass tubes and repeated again. Total fatty acids add a 100uL internal standard for the methanol fraction. Then add 500uL 1N KOH to the remaining methanol fraction, vortex and incubate for 1hr. Then add 500uL 1N HCl and check Dry down under vacuum using a speed vac. Derivative samples by adding 25 μ l 1% pentafluorobenzyl bromides in acetonitrile, and 25 μ l 1% diisopropylethylamine in acetonitrile. Cap tubes with rubber caps, vortex, and let stand at room temperature for 20 minutes. Dissolve samples in 50 μ l iso-octane and transfer to label sample vial with 250 μ l glass inserts. Cap and place samples in the GC-MS sample tray and begin analysis.

Fatty acids analysis by gas chromatography (GC)

The profile of fatty acids was completed following gas chromatographic (GC) method (Nichols *et al.*, 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 was chromatographically separated under the same conditions, using the same temperature program (oven initial temperature 140°C to final temperature 240°C, heating rate 4°C/min.), split rate 100:1. Nitrogen was invoked as a 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 subjected 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.

Result and Discussion

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 methomyl concentration of 2ppm of 30 days (79.40 \pm 0.70, 72.29 \pm 1.22 and 30.87 \pm 0.82)

when compared to another concentration of 1ppm of 30 days (81.00 \pm 0.82, 110.72 \pm 0.82 and 43.17 \pm 0.82) and control (82.60 \pm 0.80, 119.20 \pm 0.97 and 52.32 \pm 0.93) respectively. Simultaneously, lipid composition was reduced in 1ppm of 30 days (59.91 \pm 0.82) when compared to other treated groups and control, respectively (Table 1). Moisture, crude protein, and carbohydrate were decreased in methomyl 40% treated groups. 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 various tissues (Tamizhazhagan and Pugazhendy 2016) [16, 45]. The total carbohydrate level decreased has been noted in the liver and muscle of *Heteropneustes fossilis* exposed to herbicide (Sangeetha Sharma and Agarwal, 2004) [37]. Several investigators have reported a number of changes in biochemical parameters of aquatic organisms due to pesticide exposure (Remia *et al.*, 2008; Vijayakumar *et al.*, 2009 and Tamizhazhagan, 2015) [35]. Effect of toxin methomyl is 40% insecticide causes significant economic losses in the fish industry every year. Hence, contamination of the environment with methomyl based insecticides might cause serious harm to nontarget organisms. Trachantong *et al.*, (2012) [49, 50] reported that the fish play an important role in human nutrition.

The pesticides are also known to inhibit energy synthesis by suppressing aerobic oxidation of protein leading to the energy crisis in animals (Kohli *et al.*, 1975) [22]. 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 (Tamizhazhagan *et al.*, 2017) [20, 23, 31, 44, 51]. Even with the same concentration longer exposure resulted in decreased amount of protein content which indicates that the tissue protein endures proteolysis.

Toxicity data for a variety of pesticides such as organophosphate, organochlorine, carbamide and pyrethroid pesticides have been reported for a number of fish species by various author's (Gurusamy and Ramadoss, 2000; Tamizhazhagan and Pugazhendy, 2016; Sapna Shrinivasta, 2002; Nishar *et al.*, 2004 and Visvanathan *et al.*, 2009 Usha *et al.*, 2017; Jayalakshmi *et al.*, 2017; Pichaimani, *et al.*, 2017; Vijayan *et al.*, 2018) [17, 16, 45, 31, 55]. Fish proteins are well balanced with essential amino acids and are comparable to other proteins of animals origin (Tont, 1977) [46], further fish contain lipids especially omega fatty acids and free fatty acid from the human nutritious point of view. 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 *et al.*, 2013). The liver sample composition of moisture, portion, lipid and carbohydrate were decreased in the insecticide of methomyl concentration of 2ppm of 30 days (82.00 \pm 0.82, 89.95 \pm 1.02, 40.95 \pm 0.32, 59.65 \pm 1.12) while compared to another concentration of 1ppm of 30 days (82.14 \pm 0.78, 98.75 \pm 0.77, 41.15 \pm 1.02 and 62.54 \pm 0.10) and control (83.10 \pm 0.83, 133.46 \pm 0.76, 65.86 \pm 1.15 and

81.68±0.88) respectively (Table 2).

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, besides Lignoceric acid methyl ester were extant in 1ppm of 30day of methomyl 40% insecticide-treated groups only, not distinguished, while compared to other concentrated of methomyl 40% insecticide groups, as well as Palmitic acid methyl ester, was increased then compared to control, respectively (Table 3). 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 methomyl 40% insecticide-treated groups when compared to control, respectively (Table 3).

Poly Unsaturated FAs (PUFAs) as Linoleic acid methyl ester, Gamma-Linolenic acid methyl ester, Cis-8,11,14-Eicosatrienoic acid methyl ester, Cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester and Cis-13,16-Docosadienoic acid methyl ester was decline of methomyl 40% insecticide 1ppm and 2ppm treated groups, furthermore Cis-13,16-Docosadienoic acid methyl ester and Cis 4,7,10,13,16,19 Docosahexaenoic acid methyl ester fatty acid were available in methomyl 40% insecticide 1ppm and 2ppm of 30 days while compared to control, respectively (Table 3). Saturated fatty acids in muscle tissue were inferior of increased in the treated group of 1ppm of 15 days (7.403±1.044) where compared to another treatment groups of methomyl 40% insecticide (4.057±0.843, 1.25±0.218, 2.375±0.298) and control (1.377±0.156), respectively (Table 4).

Monounsaturated fatty acids were of inferior quality increased in methomyl 40% insecticide 1ppm of 30days (5.504±0.745) while compared to another treatment groups (1.389±0.258, 0.44±0.042, 0.687±0.062) and control (1.616±0.258) in muscle tissue, respectively (Table 4). Polyunsaturated fatty acids (PUFA) mean results in muscle tissue show that the expand level of methomyl 40% insecticide in 1ppm of 30 days (4.482±0.596) when compared to control (0.566±0.129) although methomyl 40% insecticide was reduced in 2ppm of 15 days (0.266±0.15) which results compared to control (0.566±0.129), respectively (Table 4).

Fatty acid and free fatty acid levels in the liver of *C. striatus*, at different periods of exposures, are presented in table 5 & 6 and figure 2. Estimation of liver tissue saturated fatty acids (SFAs) were decreased level of methomyl 40% insecticide-treated in Caproic acid methyl ester was in close proximity in 1ppm of 15 days only, Caprylic acid methyl ester, Capric acid methyl ester, Undecanoic acid methyl ester and Tricosanoic acid methyl ester was decreased in 1ppm of 15day and 1ppm of 15 and 30days respectively, methomyl 40% insecticide-treated groups, while compared to other concentrated of methomyl 40% insecticide groups and control, respectively (Table 5). 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 coenzymeA, generating acyl-CoA. The acyl-CoA is transported into the mitochondrial matrix where oxidation occurs (Sapna Shrinivasta, 2002). The 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 and Kent., 2003) ^[40], 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.

Mono Unsaturated FAs (MUFAs) liver tissue of Cis-10-Heptadecenoic acid methyl Ester, Nervonic acid methyl ester was increased in different concentration of methomyl 40% insecticide-treated in 1ppm 15days groups when compared to control, respectively (Table 5).

Polyunsaturated fatty acid as eicosapentaenoic acid (EPA, C20: 5n3) and docosahexaenoic acid (DHA, C22: 6n3) was increased, to establish a gas chromatography-mass spectrometry method, which allows precise quantification of FAs profile. The increase of EPA and DHA was also associated with a reduction of oleic acid (OA) and lauric acid (LA), but did not alter the abundance of analyzed saturated FAs. Supplementary to very numerous studies, dietary fish oil or purified PUFAs, such as EPA and DHA, are known to exert a wide range of health-beneficial effects.

Poly Unsaturated FAs (PUFAs) liver tissue of Gamma-Linolenic acid methyl ester and Cis-11,14-Eicosadienoic acid Methyl ester and Cis-8,11,14-Eicosatrienoic acid methyl ester were increased of methomyl 40% insecticide-treated in 1ppm 15days while compared to control, respectively (Table 5). Simultaneously, Arachidonic acid methyl ester was available in methomyl 40% insecticide 1ppm and 2ppm of 30 days only although not discovered to other concentration groups and then control (Table 5).

Saturated fatty acids (SFA) liver tissue was increased in 2ppm of 15 days (11.013±0.143) while compared to another treatment group of methomyl 40% insecticide and control (3.872±0.447) respectively (Table 6). Monounsaturated fatty acids were higher methomyl 40% insecticide in 2ppm of 15 days (13.549±1.490) when compared to other concentration treated groups and control (0.409±0.029) respectively (Table 6). Polyunsaturated fatty acids results show that the increased in methomyl 40% insecticide of 2ppm 15days (10.08±2.115) treated groups when compared to control (1.542±0.258) (Table 6). Saturated fatty acid as Caprylic acid methyl ester, Capric acid methyl ester, Undecanoic acid methyl ester and Tricosanoic acid methyl ester was decreased, whereas palmitic and stearic acid level increased at the end of 96hr methomyl exposure. It was interesting to 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, Montero *et al.*, (1999) ^[27] 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 methomyl 40% insecticide exposure.

This result implies that sex hormones may play important

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 quantities of fatty acids. The maximum increase in *C. striatus* liver tissue Σ MUFA (13.549 \pm 1.490) 2ppm of 15 days and muscles tissue maximum increased in Σ SFA (7.403 \pm 1.044), that compared to control, respectively (Table 4 & 6).

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

Treatment	Muscle			
	Moisture	Protein (mg/gm tissue)	Carbohydrates (mg/gm tissue)	Lipid (mg/gm tissue)
Control	82.60 \pm 0.80 ^a	119.20 \pm 0.97 ^a	52.32 \pm 0.93 ^a	75.65 \pm 0.82 ^a
Methomyl 1ppm	15 days	81.20 \pm 0.89 ^{ab}	110.72 \pm 0.82 ^b	43.17 \pm 0.82 ^b
	30 days	81.00 \pm 0.82 ^{ab}	96.21 \pm 0.96 ^c	40.20 \pm 0.99 ^c
Methomyl 2ppm	15 days	80.30 \pm 1.15 ^{ab}	83.35 \pm 0.82 ^d	42.36 \pm 1.00 ^b
	30 days	79.40 \pm 0.70 ^b	72.29 \pm 1.22 ^c	30.87 \pm 0.82 ^c

Values are given as mean \pm 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: Chemical composition of liver of *C. striatus* exposed to sub-lethal concentration of methomyl

Treatment	Liver			
	Moisture	Protein (mg/gm tissue)	Carbohydrates (mg/gm tissue)	Lipid (mg/gm tissue)
Control	83.10 \pm 0.83	133.46 \pm 0.76 ^a	65.86 \pm 1.15 ^a	12.08 \pm 0.76 ^a
Methomyl 1ppm	15 days	82.30 \pm 0.82	113.54 \pm 0.88 ^b	47.21 \pm 1.34 ^c
	30 days	82.14 \pm 0.78	98.75 \pm 0.77 ^d	41.15 \pm 1.02 ^d
Methomyl 2ppm	15 days	82.50 \pm 0.94	105.34 \pm 0.88 ^c	51.36 \pm 0.91 ^b
	30 days	82.00 \pm 0.82	89.95 \pm 1.32 ^e	40.95 \pm 1.32 ^d

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

Table 3: Fatty acid compositions in Muscles tissues of *Channa striatus* exposed to sub lethal concentrations of methomyl (40%) insecticide.

Car. Chain	Fatty acid	Control	1ppm15 days	1ppm 30 days	2ppm 15 days	2ppm 30 days
C6:0	Caproic acid methyl ester	0.263 \pm 0.111	ND	0.299 \pm 0.101	ND	0.133 \pm 0.082
C8:0	Caprylic acid methylester	0.084 \pm 0.020	0.011 \pm 0.003	0.016 \pm 0.004	0.063 \pm 0.014	0.012 \pm 0.003
C10:0	Capric acid methyl ester	0.078 \pm 0.011	0.586 \pm 0.060	0.041 \pm 0.022	ND	0.027 \pm 0.012
C11:0	Undecanoic acid methyl ester	0.016 \pm 0.001	0.073 \pm 0.008	0.058 \pm 0.032	0.083 \pm 0.019	0.039 \pm 0.014
C12:0	Lauric acid methyl ester	0.065 \pm 0.014	0.639 \pm 0.062	0.070 \pm 0.035	0.045 \pm 0.012	0.058 \pm 0.019
C13:0	Tridecanoic acid methyl ester	0.034 \pm 0.006	0.047 \pm 0.001	0.055 \pm 0.024	0.021 \pm 0.011	0.030 \pm 0.013
C14:0	Myristic acid methyl ester	0.094 \pm 0.013	0.604 \pm 0.013	0.028 \pm 0.011	0.059 \pm 0.018	0.032 \pm 0.014
C15:0	Pentadecanoic acidmethyl ester	0.185 \pm 0.040	0.852 \pm 0.028	1.456 \pm 0.254	0.050 \pm 0.015	0.595 \pm 0.142
C16:0	Palmitic acid methyl ester	0.047 \pm 0.008	2.528 \pm 0.258	0.058 \pm 0.034	0.643 \pm 0.190	0.048 \pm 0.015
C17:0	Heptadecanoic acid methyl ester	0.311 \pm 0.121	0.071 \pm 0.005	1.557 \pm 0.260	0.039 \pm 0.016	1.198 \pm 0.317
C18:0	Stearic acid methyl ester	0.031 \pm 0.005	1.839 \pm 0.147	0.120 \pm 0.098	0.121 \pm 0.038	0.028 \pm 0.012
C20:0	Arachidic acid methyl ester	0.021 \pm 0.002	ND	0.097 \pm 0.065	ND	0.015 \pm 0.010
C21:0	Henicosanoic acid methyl ester	0.035 \pm 0.006	0.058 \pm 0.007	0.076 \pm 0.045	0.038 \pm 0.016	0.036 \pm 0.013
C22:0	Behenic acid methyl ester	0.019 \pm 0.002	ND	0.029 \pm 0.011	ND	0.021 \pm 0.011
C23:0	Tricosanoic acid methyl ester	0.094 \pm 0.018	0.095 \pm 0.003	0.080 \pm 0.050	0.088 \pm 0.023	0.103 \pm 0.028
C24:0	Lignoceric acid methyl ester	ND	ND	0.017 \pm 0.010	ND	ND
Σ SFA		1.377	7.403	4.057	1.25	2.375
C14:1	Myristoleic acid methyl ester	0.042 \pm 0.011	0.081 \pm 0.005	0.016 \pm 0.001	0.026 \pm 0.011	ND
C15:1	Cis-10-Pentadecenoic acid methyl Ester	0.694 \pm 0.109	0.810 \pm 0.079	0.092 \pm 0.061	0.170 \pm 0.103	0.047 \pm 0.026
C16:1	Palmitoleic acid methyl ester	0.072 \pm 0.010	0.044 \pm 0.007	0.020 \pm 0.011	0.012 \pm 0.001	ND
C17:1	Cis-10-Heptadecenoic acid methyl Ester	0.669 \pm 0.115	ND	0.033 \pm 0.015	ND	0.028 \pm 0.010
C18:1n9t	Elaidic acid methyl ester	0.076 \pm 0.010	ND	4.799 \pm 0.897	0.169 \pm 0.103	0.484 \pm 0.124
C18:1n9c	Oleic acid methyl ester	0.032 \pm 0.009	0.174 \pm 0.026	0.018 \pm 0.011	0.063 \pm 0.014	0.015 \pm 0.001
C20:1n9	Cis-11-Eicosenoic acid methyl Ester	0.031 \pm 0.006	ND	0.236 \pm 0.092	ND	0.113 \pm 0.027
C22:1n9	Erucic acid methyl ester	ND	ND	0.150 \pm 0.093	ND	ND
C24:1n9	Nervonic acid methyl ester	ND	0.280 \pm 0.049	0.140 \pm 0.095	ND	ND
Σ MUFA		1.616	1.389	5.504	0.44	0.687
C18:2n6t	Linolelaidic acid methyl ester	0.212 \pm 0.035	0.623 \pm 0.073	0.223 \pm 0.095	0.014 \pm 0.006	0.477 \pm 0.126
C18:2n6c	Linolenic acid methyl ester	0.014 \pm 0.002	0.064 \pm 0.005	0.022 \pm 0.011	0.042 \pm 0.015	\pm

C18:3n3	Alpha-Linolenic acid methyl ester	0.022±0.001	ND	0.050±0.018	ND	0.029±0.011
C18:3n6	Gamma-Linolenic acid methyl Ester	0.165±0.030	0.415±0.091	0.960±0.019	0.034±0.015	0.037±0.010
C20:2	Cis-11,14-Eicosadienoic acid Methyl ester	0.034±0.002	0.092±0.003	0.372±0.086	0.043±0.016	0.095±0.026
C20:3n6	Cis-8,11,14-Eicosatrienoic acid methyl ester	0.077±0.004	0.074±0.004	0.053±0.019	0.040±0.014	0.030±0.012
C20:3n3	Cis-11,14,17-Eicosatrienoic acid methyl ester	ND	0.112±0.054	0.607±0.162	ND	0.105±0.034
C20:5n3	Cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester	0.042±0.004	1.562±0.258	0.021±0.012	0.093±0.023	0.041±0.031
C22:2	Cis-13,16-Docosadienoic acid methyl ester	ND	ND	0.071±0.021	ND	0.140±0.038
C22:6n3	Cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester	ND	ND	2.103±0.517	ND	0.275±0.053
ΣPUFA		0.566	2.942	4.482	0.266	1.229

Values are given as mean±SE. ND-Not detected

Table 4: Free fatty acids profile in muscle tissue of *C. striatus* exposed to sub-lethal concentration of (methomyl 40%) insecticide.

Treatment	Muscle		
	ΣSFA	ΣMUFA	ΣPUFA
Control	1.377±0.156 ^c	1.616±0.258 ^b	0.566±0.129 ^{cd}
Methomyl 1ppm	15 days	7.403±1.044 ^a	2.942±0.299 ^b
	30 days	4.057±0.843 ^b	4.482±0.596 ^a
Methomyl 2ppm	15 days	1.25±0.218 ^c	0.266±0.015 ^e
	30 days	2.375±0.298 ^{ab}	1.229±0.149 ^c

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

Table 5: Fatty acid compositions in Liver tissues of *C. striatus* exposed to sub-lethal concentrations of methomyl (40%) insecticide.

Carbon Chain	Fatty acid	Control	Methomyl 1ppm	Methomyl 1ppm	Methomyl 2ppm	Methomyl 2ppm
			15 days	30 days	15 days	30 days
C6:0	Caproic acid methyl ester	0.209±0.024	0.178±0.101	ND	ND	ND
C8:0	Caprylic acid methylester	0.014±0.003	0.011±0.002	0.178±0.101	0.674±0.232	0.624±0.106
C10:0	Capric acid methyl ester	0.065±0.021	0.034±0.012	0.028±0.028	0.890±0.353	0.659±0.098
C11:0	Undecanoic acid methyl ester	0.074±0.010	0.090±0.058	0.772±0.131	1.567±0.423	0.05±0.006
C12:0	Lauric acid methyl ester	0.054±0.011	0.116±0.082	0.037±0.014	0.331±0.121	0.184±0.026
C13:0	Tridecanoic acid methyl ester	0.068±0.012	0.032±0.012	0.013±0.007	0.394±0.119	0.026±0.007
C14:0	Myristic acid methyl ester	0.052±0.021	0.031±0.011	0.206±0.061	0.572±0.181	0.374±0.071
C15:0	Pentadecanoic acidmethyl ester	1.127±0.337	0.620±0.023	0.223±0.034	0.330±0.123	0.171±0.026
C16:0	Palmitic acid methyl ester	0.066±0.010	0.049±0.022	0.124±0.081	ND	2.580±0.494
C17:0	Heptadecanoic acid methyl ester	1.875±0.260	1.228±0.201	0.054±0.020	5.345±1.089	0.117±0.036
C18:0	Stearic acid methyl ester	0.070±0.012	0.072±0.037	0.314±0.084	0.400±0.120	2.559±0.516
C20:0	Arachidic acid methyl ester	0.058±0.018	0.069±0.037	0.029±0.010	ND	0.043±0.007
C21:0	Henicosanoic acid methyl ester	0.044±0.019	0.054±0.027	0.171±0.030	ND	0.054±0.006
C22:0	Behenic acid methyl ester	0.016±0.001	0.023±0.010	0.018±0.010	ND	0.0451±0.008
C23:0	Tricosanoic acid methyl ester	0.080±0.042	0.080±0.047	0.116±0.029	0.510±0.175	0.089±0.013
C24:0	Lignoceric acid methyl ester	ND	0.017±0.010	0.020±0.011	-----	0.039±0.007
ΣSFA		3.872	2.704	2.303	11.013	7.614
C14:1	Myristoleic acid methyl ester	0.017±0.003	0.017±0.011	0.027±0.011	1.560±0.518	0.017±0.004
C15:1	Cis-10-Pentadecenoic acid methyl Ester	0.077±0.024	0.064±0.031	0.333±0.119	0.240±0.116	0.054±0.006
C16:1	Palmitoleic acid methyl ester	0.020±0.006	0.017±0.011	0.013±0.001	9.520±1.648	0.107±0.026
C17:1	Cis-10-Heptadecenoic acid methyl Ester	0.055±0.023	0.072±0.038	0.084±0.038	ND	0.025±0.007
C18:1n9t	Elaidic acid methyl ester	ND	0.523±0.050	0.179±0.061	ND	0.272±0.052
C18:1n9c	Oleic acid methyl ester	0.053±0.027	0.014±0.001	0.023±0.010	1.389±0.369	0.054±0.007
C20:1n9	Cis-11-Eicosenoic acid methyl Ester	0.055±0.018	0.075±0.045	ND	ND	ND
C2 2:1n9	Erucic acid methyl ester	0.069±0.030	0.6+057±0.030	0.024±0.012	ND	ND
C24:1n9	Nervonic acid methyl ester	0.063±0.031	0.105±0.020	0.038±0.018	0.840±0.183	0.431±0.073
ΣMUFA		0.409	0.944	0.721	13.549	0.96
C18:2n6t	Linolelaidic acid methyl ester	0.027±0.016	0.060±0.021	0.017±0.010	2.580±0.423	0.031±0.008
C18:2n6c	Linolenic acid methyl ester	0.050±0.020	0.062±0.033	0.075±0.032	3.490±0.775	0.027±0.006
C18:3n3	Alpha-Linolenic acid methyl ester	0.029±0.012	ND	ND	ND	0.360±0.077
C18:3n6	Gamma-Linolenic acid methyl Ester	0.045±0.022	0.052±0.022	0.017±0.010	0.550±0.183	0.028±0.006
C20:2	Cis-11,14-Eicosadienoic acid Methyl ester	0.168±0.096	0.230±0.036	0.036±0.014	0.480±0.238	0.045±0.010
C20:3n6	Cis-8,11,14-Eicosatrienoic acidmethyl ester	0.044±0.019	0.255±0.118	0.018±0.011	1.200±0.264	0.031±0.008
C20:3n3	Cis-11,14,17-Eicosatrienoic acid methylester	0.195±0.093	0.186±0.094	0.028±0.011	0.790±0.186	ND
C20:5n3	Cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester	0.100±0.068	0.052±0.022	0.142±0.046	0.990±0.183	0.541±0.080
C22:2	Cis-13,16-Docosadienoic acid methyl ester	0.034±0.018	0.101±0.078	0.487±0.119	ND	ND
C22:6n3	C Cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester	0.850±0.151	0.900±0.163	0.049±0.027	ND	ND

C24:0	Lignoceric acid methyl ester	ND	ND	ND	ND	0.039±0.007
C20:4n6	Arachidonic acid methyl ester	ND	ND	0.027±0.011	ND	0.441±0.072
ΣPUFA		1.542	1.898	0.896	10.08	1.543

Values are given as mean±SE, ND- Not detected.

Table 6: Free fatty acids profile in liver tissue of *C. striatus* exposed to sub-lethal concentration of (methomyl 40%) insecticide.

Treatment		Muscle		
		ΣSFA	ΣMUFA	ΣPUFA
Control		3.872±0.447 ^c	0.409±0.029 ^b	1.542±0.258 ^b
Methomyl 1ppm	15 days	2.704±0.421 ^d	0.944±0.083 ^b	1.898±0.365 ^b
	30 days	2.303±0.857 ^e	0.721±0.093 ^b	0.896±0.137 ^b
Methomyl 2ppm	15 days	11.013±0.143 ^a	13.549±1.490 ^a	10.08±2.115 ^a
	30 days	7.614±0.336 ^b	0.96±0.725 ^b	1.543±0.258 ^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).

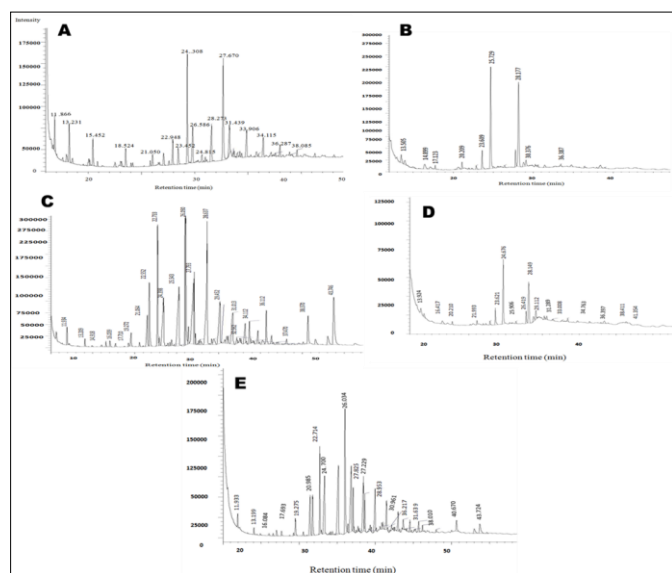


Fig 1: Muscle RT

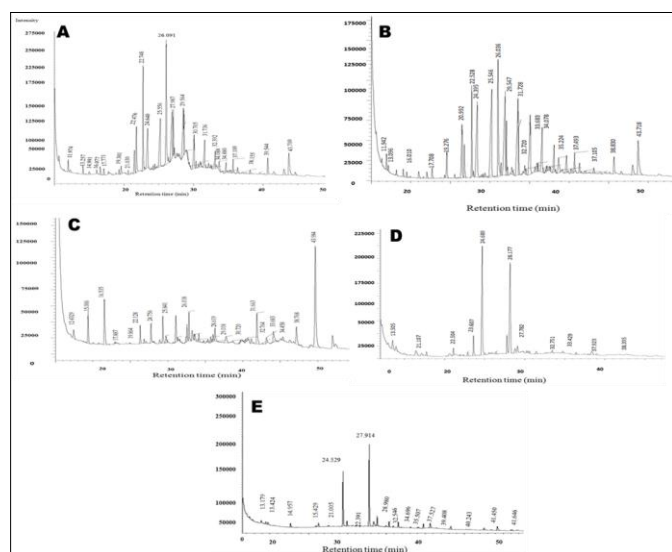


Fig 2: Liver RT

Conclusion

In this investigation exposed that sub-lethal effect of methomyl 40% on chemical composition and fatty acid profile

of *C. striatus*. Environmental methomyl 40% exposure can cause changes in the fatty acid composition of *C. striatus* muscle and liver tissue. With increased concentration of methomyl 40% 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 methomyl 40%-concentration dependent.

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