

FATTYACID PROFILING OF ZEBRAFISH TESTIS TREATED WITH FIPRONIL

(PROJECT – REPORT)

Submitted in partial fulfilment of the requirements for the award of

Master of Science

In

Medical Biotechnology and Clinical Research

by

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CENTRE FOR MOLECULAR AND NANOMEDICAL SCIENCES

INTERNATIONAL RESEARCH CENTRE

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with grade 'A' by NAAC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **MAHALAKSHMI.P (41101008)** and who carried out the project entitled **"FATTYACID PROFILING OF ZEBRAFISH TESTIS TREATED WITH FIPRONIL"** under our supervision from December 2022 to May 2023.

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DECLARATION

I **MAHALAKSHMI.P(41101008)** hereby declare that the project report entitled **"FATTYACID PROFILING OF ZEBRAFISH TESTIS TREATED WITH FIPRONIL"** done by me under the guidance of **Dr. R.RAJESH KANNAN** at **Sathyabama institute of Science and Technology, Chennai** is submitted in partial fulfilment of the requirements for the award of Master of science degree in Medical Biotechnology and clinical research.

DATE: 05.05.2023

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ABSTRACT

Fipronil is an insecticide widely used in agriculture that can be harmful to aquatic organisms, including fish. This study aimed to investigate the effects of fipronil on the fatty acid profile of zebrafish testis. To evaluate the effects of fipronil on vertebrates, we used zebrafish as an animal model. This study aimed to investigate the impact of fipronil exposure on the fatty acid composition of zebrafish testis. Testis were collected from adult zebrafish exposed to varying concentrations (10, 25, 50, 100 and 300 µg/lit) of fipronil for 72 hrs, and lipid extracts were analysed using gas chromatography-mass spectrometry.

Key words : Fipronil, Insecticide, zebrafish testis, fatty acid, gas chromatography – mass spectrometry

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LIST OF ABBREVIATION AND SYMBOLS

FDA- Food and Drug Administration

FSH- Follicle-stimulating hormone

LH- Luteinizing hormone

ROS- Reactive oxygen species

SOD- Superoxide dismutase

NaCl-Sodium chloride

NaOH-Sodium hydroxide

H₂SO₄-Sulphuric acid

GC-gas chromatography

MS-Mass spectrometers

µg/L /L-Microgram per litre

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INTRODUCTION

In 1993, Rhône-Poulenc introduced the pesticide fipronil to the market after discovering and developing it between 1985 and 1987. Although it works well against many different pests, there are worries about how it will affect the environment and people's health. An effective low-rate pesticide is fipronil. The material's molecular mass is 437, and it has a white powdery appearance and musty smell. It is only slightly soluble in water and is stable for a year at room temperature, but it becomes unstable when metal ions are present. It is broken down by sunlight into several different metabolites, one of which (fipronil-desulfinyl) is exceptionally stable and more poisonous than the parent chemical is widely used in agriculture for soil treatment and seed coating (colin.c. tingle et.al 2003). A new class of insecticides known as phenylpyrazoles includes fipronil, it is the (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1H-pyrazole] in terms of chemistry. Frontline, a well-known ectoparasiticide, contains the active component fipronil. Other ectoparasiticide products, such Certifect and Parastar Plus, also include fipronil. There are now two commercially available versions of Frontline preparation for dogs and cats (a spray and a spot-on topical application). The item is designed to eliminate fleas, mites, brown dog ticks, American dog ticks, lone star ticks, which may carry Lyme disease, and all phases of these ticks (Cutler, 1998; vbHutchinson et al., 1998; Anonymous, 2000).

Fipronil is used to control several insects, including ants, beetles, cockroaches, fleas, ticks, termites, mole crickets, thrips, rootworms, and weevils. Each fipronil product has a different set of uses. When using pesticides, always read and abide by the label. In addition to being utilised in agriculture, fipronil is also employed in granular turf products, seed treatments, topical pet care products, gel baits, and liquid termiticides. Caution to danger are possible signal phrases for fipronil-containing products. The warning phrase represents the product's overall toxicity when the active ingredient and other chemicals are used together (Jackson, D et.al 2009).

In aerobic soils, fipronil has a half-life of 122-128 days. Fipronil is broken down by naturally occurring soil organisms into fipronil-sulfone under aerobic conditions. Fipronil can hydrolyze to create fipronil-amide as well. See the Half-life text box. Fipronil has a measured half-life of 34 days in loamy soil and breaks down on soil surfaces when exposed to UV radiation, or sunlight. However, in field conditions, soil particles may prevent light from penetrating any appreciable layer of soil and hence lengthen residence time.

In water, fipronil quickly breaks down into fipronil-desulfinyl when exposed to UV radiation. Fipronil has a half-life of between 4 and 12 hours in these circumstances. At pH 5 and pH 7, fipronil is resistant to hydrolysis. It deteriorates, however, in direct proportion to rising pH levels in alkaline circumstances.

The United States Food and Drug Administration (FDA) Pesticide Residue Monitoring Programme carries out regulatory and incidence/level monitoring for pesticide residues in domestic and imported foods (apart from meat, poultry, dairy, and eggs). In 2003, the FDA examined 84 domestic samples (3.6% of domestic samples) for levels of fipronil for tolerance compliance. Fipronil was not found in any samples in detectable amounts. The FDA examined more than 150 samples of imported food in 2003 to check for fipronil levels. Fipronil residue levels that were over the threshold were found in two samples. Pesticide residues in meat, poultry, dairy, and eggs are subject to regulatory monitoring by the Department of Agriculture (USDA). The USDA examined 655 samples of chicken thighs and breasts in 2006 to check for the presence of fipronil. Fipronil was found in one chicken breast (0.2%) and two chicken thighs (0.3%).

After using fipronil, humans have reported experiencing tonic-clonic seizures, sweating, nausea, vomiting, headaches, abdominal discomfort, dizziness, and agitation. Fipronil-related clinical symptoms typically resolve on their own and are reversible (Jackson et al. 2009).

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Table 1: Residue levels of fipronil in the market fruits and vegetables

F/V	SUBSTRATE	HR
F	Dragon fruit	0.0262
V	Eggplant	0.0095
V	Tomato	0.000001
V	Cabbage	0.0029
F	Plum	0.0018
V	Lettuce	1.86
F	Grape	0.0056
V	bean	1.0026
V	Water spinach	0.1005
V	Sweet potato	0.0144

F/V : fruit/vegetables, HR :highest residue concentration



Fig.1 Zebrafish-danio rerio

The latin name "Danio rerio" is used to refer to zebrafish. They go by the name Zebrafish because of their striped bodies, which resemble those of a zebra. From

the caudal fin to their gills, the body of the fish is covered with horizontal, navy-colored stripes. Although zebrafish have long been maintained as pets, George Streisinger utilised them for the first time in 1960s for medical research. After years of effort, Streisinger was able to successfully clone a zebrafish, producing the first successful vertebrate clone ever. Due to their many advantageous characteristics, zebrafish are frequently employed in research.

First of all, zebrafish are highly durable and simple to keep. Since ponds are where they typically live, it is simple to recreate the optimal environment for them. They are also quite affordable, however they take up more room than other model organisms like flies. This model has an advantage over other models in that it is a vertebrate as well.

Zebrafish have extraordinarily high fecundity, producing multiple hundreds of eggs each week. They also have a very short generation time, meaning that scientists have a never-ending supply of this model. This shortens the overall experimental process and is particularly useful in mutant production. Another advantage is that Zebrafish eggs develop externally, allowing scientists to study their initial development easily. This is an advantage over other models, such as mice, which develop internally and therefore hinder visual investigation.

TAXONOMY

- Kingdom: Animalia
- Phylum: Chordata
- Subphylum: Vertebrata
- Class: Actinopterygii
- Order: Cypriniformes
- Family: Cyprinidae
- Genus: Danio
- Species: rerio

CHAPTER – 2

REVIEW OF LITERATURE

Javed M et al. (2021) investigates the effects of the insecticide fipronil on the reproductive and endocrine functions in male zebrafish (*Danio rerio*) using both in vivo and in silico approaches. In the in vivo experiment, male zebrafish were exposed to different concentrations of fipronil for 21 days, after which their reproductive and endocrine functions were assessed. The results showed that exposure to fipronil caused a decrease in the levels of testosterone and follicle-stimulating hormone (FSH), as well as a decrease in the size of testes and the number of spermatozoa in the epididymis. In addition, fipronil exposure led to an increase in the expression of genes related to the synthesis of estradiol. In the in silico approach, molecular docking studies were conducted to investigate the interaction of fipronil with the androgen receptor (AR) and the estrogen receptor (ER). The results showed that fipronil has a higher affinity for the ER than for the AR, suggesting that fipronil may act as an estrogenic compound and disrupt the endocrine system in male zebrafish. Overall, the study suggests that fipronil exposure can disrupt the reproductive and endocrine functions in male zebrafish, and highlights the importance of assessing the potential endocrine disrupting effects of pesticides and other chemicals.

Waleed.A et al.(2020) This study showed that exposure to fipronil led to significant histological alterations in the testes and epididymis of the zebrafish, as well as a decrease in sperm quality. The researchers also found that fipronil exposure altered the expression of genes involved in reproductive function. However, when quercetin was administered along with fipronil, it partially alleviated the negative effects of the insecticide on the reproductive system of the fish. Quercetin treatment improved the histological structure of the testes and epididymis and increased sperm quality. It also upregulated the expression of genes related to reproductive function. Overall, the study suggests that fipronil can have detrimental effects on the reproductive system of male zebrafish, but quercetin may have a protective effect. The findings highlight the importance of studying the effects of environmental toxins on aquatic life and the potential use of natural compounds to mitigate their negative impacts.

Zhang X et al.(2020) This study showed that chronic exposure to fipronil led to significant histological alterations in the testes of the zebrafish, including a decrease in the number and size of spermatogonia and spermatozoa. The researchers also observed a reduction in sperm motility and viability, as well as an increase in

abnormal sperm morphology. These findings indicate that fipronil exposure may cause reproductive toxicity in male zebrafish. In addition, the study found that chronic exposure to fipronil caused endocrine disruption in male zebrafish by altering the expression of genes related to the hypothalamic-pituitary-gonadal axis. Specifically, the researchers observed a decrease in the expression of genes involved in testosterone synthesis and spermatogenesis. Overall, the study suggests that chronic exposure to fipronil may have negative effects on the reproductive system and endocrine function in male zebrafish. The findings highlight the potential risks associated with the widespread use of fipronil in agriculture and the importance of further research on the effects of environmental toxins on aquatic life.

Sun L et al.(2019) The study showed that fipronil exposure caused a reduction in testosterone levels and a decrease in the expression of genes involved in testosterone synthesis and regulation. This suggests that fipronil exposure may disrupt the endocrine system of male zebrafish by interfering with testosterone synthesis and regulation. In addition, the study found that fipronil exposure led to histological alterations in the testes of the zebrafish, including a decrease in the number of spermatogonia and spermatozoa. The researchers also observed a reduction in sperm motility and viability, as well as an increase in abnormal sperm morphology, indicating potential reproductive toxicity. Furthermore, the researchers identified several key molecular pathways involved in the endocrine disrupting effects of fipronil, including the estrogen receptor signaling pathway and the JAK/STAT signalling pathway. These findings suggest that fipronil may disrupt the endocrine system of male zebrafish through multiple molecular mechanisms. Overall, the study provides evidence for the potential endocrine disrupting effects of fipronil on male zebrafish and highlights the need for further research on the effects of environmental toxins on aquatic life.

Guo J et al.(2018) In this study fipronil exposure during the embryonic and larval stages caused a delay in male sexual development and a decrease in testosterone levels. Additionally, fipronil exposure during the adult stage led to a decrease in sperm motility and viability, as well as an increase in abnormal sperm morphology. The study also found that fipronil exposure altered the expression of genes related to reproductive development and endocrine function, including genes involved in testosterone synthesis and regulation. Specifically, the researchers

observed a decrease in the expression of genes related to steroidogenesis and sperm maturation, as well as an increase in the expression of genes related to estrogen synthesis and metabolism. Overall, the study provides evidence for the potential endocrine disrupting effects of fipronil on the development and reproduction of male zebrafish, and highlights the need for further research on the effects of environmental toxins on aquatic life. The findings also suggest that fipronil exposure may alter the expression of genes involved in both androgen and estrogen synthesis and regulation, potentially leading to a disruption of the balance between male and female hormones in male zebrafish.

Sun J et al.(2017) In this study they exposed male zebrafish to fipronil at concentrations of 1, 10, and 100 µg/L for 28 days. They found that chronic exposure to fipronil resulted in a decrease in the testicular index, which is an indicator of testicular weight relative to body weight, indicating testicular damage. In addition, the sperm quality was reduced, including a decrease in sperm motility and abnormal sperm morphology. Moreover, the researchers found that chronic exposure to fipronil altered the levels of several hormones involved in reproductive function, including testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). They observed a decrease in testosterone levels and an increase in FSH and LH levels in male zebrafish exposed to fipronil. Overall, this study provides evidence that chronic exposure to fipronil can adversely affect the reproductive system of male zebrafish by altering hormone levels and causing testicular damage.

Yin L et al.(2017) In this study, male zebrafish were exposed to fipronil at concentrations of 0.1, 1, and 10 µg/L for 21 days. The researchers found that fipronil exposure caused a significant delay in testis maturation, as indicated by the reduced number of spermatogonia and increased number of spermatocytes. Moreover, the production of steroidogenic hormones, including testosterone, 11-ketotestosterone, and 17β-estradiol, was significantly reduced in fipronil-exposed male zebrafish. Furthermore, the researchers found that fipronil exposure resulted in a decrease in the expression of several genes involved in steroidogenesis, including *cyp19a1a*, *cyp11b*, and *hsd3b*. These genes encode enzymes that are essential for the synthesis of testosterone and other steroid hormones. Overall, this study provides evidence that fipronil exposure can disrupt gonadal maturation and steroidogenic hormone production in male zebrafish by affecting the expression of key genes

involved in steroidogenesis. These findings suggest that fipronil exposure may have adverse effects on the reproductive health of aquatic organisms.

Shao Y et al. (2016) investigated the effects of subchronic exposure to fipronil on oxidative stress and histopathological changes in the liver and kidney of male zebrafish. Fipronil is a widely used insecticide that has been detected in various aquatic environments. male zebrafish were exposed to fipronil at concentrations of 1, 10, and 100 µg/L for 28 days. The researchers found that fipronil exposure caused oxidative stress in the liver and kidney of male zebrafish, as evidenced by the significant increase in reactive oxygen species (ROS) levels and the decrease in antioxidant enzyme activities, including superoxide dismutase (SOD) and catalase (CAT). Moreover, fipronil exposure resulted in histopathological changes in the liver and kidney of male zebrafish, including hepatocyte vacuolization, inflammatory cell infiltration, and renal tubular epithelial cell necrosis. Furthermore, the researchers found that fipronil exposure caused a significant increase in the expression of genes related to oxidative stress and inflammation, including *sod1*, *cat*, and *tnfa*. These findings suggest that fipronil exposure can induce oxidative stress and inflammation in male zebrafish, leading to histopathological changes in the liver and kidney. Overall, this study provides evidence that subchronic exposure to fipronil can have adverse effects on the liver and kidney of male zebrafish by inducing oxidative stress, inflammation, and histopathological changes. These findings suggest that fipronil exposure may have a negative impact on the health of aquatic organisms and the ecological balance of aquatic ecosystems.

Wang C et al. (2016) investigated the effects of fipronil on apoptosis and mitochondrial pathways in male germ cells of zebrafish. Fipronil is a widely used insecticide that has been detected in various aquatic environments. male zebrafish were exposed to fipronil at concentrations of 1, 10, and 100 µg/L for 21 days. The researchers found that fipronil exposure resulted in a significant increase in apoptosis in male germ cells, as indicated by the increased number of TUNEL-positive cells. Moreover, the researchers observed that fipronil exposure resulted in a significant increase in the expression of genes related to apoptosis, including caspase-3, caspase-9, *bax*, and cytochrome *c*. In addition, fipronil exposure caused a decrease in the expression of the anti-apoptotic gene *bcl-2*. These findings suggest that fipronil exposure can induce apoptosis in male germ cells of zebrafish

through the activation of caspase-dependent mitochondrial pathways. Furthermore, the researchers found that fipronil exposure resulted in a significant decrease in the expression of genes related to mitochondrial function, including *nd2*, *cox1*, and *atp6*. These genes are essential for mitochondrial function and energy metabolism. Overall, this study provides evidence that fipronil exposure can induce apoptosis in male germ cells of zebrafish through caspase-dependent mitochondrial pathways and can also affect mitochondrial function and energy metabolism. These findings suggest that fipronil exposure may have adverse effects on the reproductive health of aquatic organisms.

Zhang Y et al. (2016) investigated the effects of fipronil on oxidative stress and apoptosis in male germ cells of zebrafish. Fipronil is a widely used insecticide that has been detected in various aquatic environments. male zebrafish were exposed to fipronil at concentrations of 1, 10, and 100 µg/L for 28 days. The researchers found that fipronil exposure resulted in a significant increase in oxidative stress in male germ cells, as evidenced by the increase in ROS levels and the decrease in antioxidant enzyme activities, including SOD and CAT. Moreover, the researchers observed that fipronil exposure resulted in a significant increase in apoptosis in male germ cells, as indicated by the increased number of TUNEL-positive cells. The researchers also found that fipronil exposure resulted in a significant increase in the expression of genes related to apoptosis, including caspase-3, caspase-9, *bax*, and cytochrome *c*. In addition, fipronil exposure caused a decrease in the expression of the anti-apoptotic gene *bcl-2*. These findings suggest that fipronil exposure can induce oxidative stress and apoptosis in male germ cells of zebrafish. Furthermore, the researchers found that fipronil exposure resulted in a significant decrease in the expression of genes related to spermatogenesis, including *c-kit* and *sox9b*. These genes are essential for male germ cell proliferation and differentiation. Overall, this study provides evidence that fipronil exposure can induce oxidative stress and apoptosis in male germ cells of zebrafish and can also affect genes related to spermatogenesis. These findings suggest that fipronil exposure may have adverse effects on the reproductive health of aquatic organisms.

CHAPTER -3

AIM AND SCOPE

3.1 AIM :

To evaluate the changes in fatty acids for zebrafish testis which is treated with fipronil by gas chromatography

3.2 OBJECTIVE :

1. Zebrafish treated with fipronil in five different concentrations (10, 25, 50, 100 and 300 µg/L)
2. Testis were dissected in four time intervals (24hrs, 48hrs and 72hrs)
3. Gas chromatography – lipid profiling
4. Concentration of lipids were observed and tabulated

CHAPTER – 4

MATERIALS AND METHODOLOGY

4.1 MATERIALS :

- Fish tank

SPECIMEN :

- male Fish

CHEMICALS:

- Fipronil
- Tricaine methane sulfonate solution
- Methanol
- Chloroform
- 1 % NaCl
- 1% Methanolic NaOH
- 1M Methanolic H₂SO₄
- Heptane

INSTRUMENTS:

- Vortex
- Centrifuge
- Pipette
- Rotary evaporator
- Gas chromatography – Mass spectrometry

4.2 METHODOLOGY:

4.2.1 CHEMICAL PREPARATION :

FIPRONIL :

It is bought from an agriculture pesticide dealer; It consist of 80% fipronil and rest 20% are solvents

Solvents :

sodium polycarboxylate(2.90%)

sodium dioctyl sulfosuccinate(1.90%)

silicone oil emulsion (0.30%)

sodium lignosulphonate(Q.S.)

PREPARATION:

1g of fipronil is dissolved in 1 litre of water

TRICAINE METHANE SULFONATE :

- Tricaine powder – 400mg
- Double distilled water – 97.9ml
- 1M Tris – 2.1ml

400 mg of tricaine powder is dissolved in 97.9ml double distilled water and 1M Tris

1% SODIUM CHLORIDE (NaCl)

- NaOH – 1g
- Distilled water – 100ml

1g of NaOH is dissolved in 100ml of distilled water

1% METHANOLIC SODIUM HYDROXIDE(NaOH)

- Methanol – 500ml
- NaOH – 5g

5g of NaOH is dissolved in 500ml of methanol

1M METHANOLIC SULFURIC ACID(H₂SO₄)

- Methanol – 500ml
- Sulfuric acid – 27ml

27ml of Sulfuric acid dissolved in 500ml of methanol

GAS CHROMATOGRAPHY

PRINCIPLE:

gas chromatography (GC) is based on the separation of a mixture of compounds into individual components by partitioning them between a stationary phase and a mobile phase. The stationary phase is typically a solid support material coated with a thin film of a liquid phase or a polymer, while the mobile phase is an inert gas such as helium or nitrogen.

The separation occurs due to differences in the affinity of the components for the stationary and mobile phases. As the mixture is injected into the GC column, the

mobile phase carries the sample through the column, and the components interact with the stationary phase to varying degrees. The components that have a higher affinity for the stationary phase will move more slowly through the column, while those with a higher affinity for the mobile phase will move more quickly.

As the components exit the GC column, they pass through a detector, which produces a signal that is proportional to the concentration of each component. The most commonly used detectors in GC are flame ionization detectors (FID) and mass spectrometers (MS).

The FID measures the concentration of compounds by detecting the ions that are generated when the sample molecules are burned in a hydrogen flame. The MS detector ionizes the sample molecules and separates them based on their mass-to-charge ratio, generating a mass spectrum that provides a unique fingerprint for each compound.

The data generated by the detector is analysed to identify and quantify the individual components in the mixture. GC is widely used in many fields, including environmental monitoring, forensic analysis, pharmaceuticals, and food and flavor analysis, due to its ability to separate and identify complex mixtures of compounds with high sensitivity and specificity.

COMPONENTS

Carrier gas

Helium, N₂, H, Argon are used as carrier gases.

Helium is preferred for thermal conductivity detectors because of its high thermal conductivity relative to that of most organic vapors.

N₂ is preferable when a large consumption of carrier gas is employed.

Carrier gas from the tank passes through a toggle valve, a flow meter, (1-1000 ml/min), capillary restrictors, and a pressure gauge (1-4 atm).

Flow rate is adjusted by means of a needle valve mounted on the base of the flow meter and controlled by capillary restrictors.

The operating efficiency of the gas chromatograph is directly dependant on the maintenance of constant gas flow.

Sample injector

Liquid samples are injected by a microsyringe with a needle inserted through a self-sealing, silicon-rubber septum into a heated metal block by a resistance heater.

Gaseous samples are injected by a gas-tight syringe or through a by-pass loop and valves.

Typical sample volumes range from 0.1 to 0.2 ml.

The separation column

The heart of the gas chromatography is the column which is made of metals bent in U shape or coiled into an open spiral or a flat pancake shape.

Copper is useful up to 250°

Swege lock fittings make column insertion easy.

Several sizes of columns are used depending upon the requirements.

Stationary phases

An infinite variety of liquid phases are available limited only by their volatility, thermal stability and ability to wet the support.

No single phase will serve for all separation problems at all temperatures.

Non-Polar – Parafin, squalane, silicone greases, apiezon L, silicone gum rubber. These materials separate the components in order of their boiling points.

Intermediate Polarity – These materials contain a polar or polarizable group on a long non-polar skeleton which can dissolve both polar and non-polar solutes. For example. diethyl hexyl phthalate is used for the separation of high boiling alcohols.

Polar – Carbowaxes – Liquid phases with a large proportion of polar groups. Separation of polar and non-polar substances.

Hydrogen bonding – Polar liquid phases with high hydrogen bonding e.g. Glycol.

Specific purpose phases – Relying on a chemical reaction with solute to achieve separations. e.g AgNO₃ in glycol separates unsaturated hydrocarbons.

Supporting material

The structure and surface characteristics of the support materials are important parameters, which determine the efficiency of the support and the degree of separation respectively.

The support should be inert but capable of immobilizing a large volume of liquid phase as a thin film over its surface.

The surface area should be large to ensure the rapid attainment of equilibrium between stationary and mobile phases.

Support should be strong enough to resist breakdown in handling and be capable of packed into a uniform bed.

Diatomaceous earth, kieselguhr treated with Na_2CO_3 for 900°C causes the particle fusion into coarser aggregates.

Glass beads with a low surface area and low porosity can be used to coat up to 3% stationary phases.

Porous polymer beads differing in the degree of cross-linking of styrene with alkyl-vinyl benzene are also used which are stable up to 250°

Detector

Detectors sense the arrival of the separated components and provide a signal.

These are either concentration-dependent or mass dependant.

The detector should be close to the column exit and the correct temperature to prevent decomposition.

Recorder

The recorder should be generally 10 mv (full scale) fitted with a fast response pen (1 sec or less). The recorder should be connected with a series of good quality resistances connected across the input to attenuate the large signals.

An integrator may be a good addition.

4.2.3 SAMPLE PREPARATION : Male zebrafish was treated in five different concentration (10,25,50,100 and $300\mu\text{g/L}$).



Fig..2 Fish treated in different concentration

Testis of the zebrafish were dissected in four different time intervals i.e. 24hrs,48 hrs and 72hrs)



Fig.3 Fish exposed to tricaine

4.2.4 TISSUE PROCESSING :

The tissue(testis) is homogenized with chloroform and methanol in 2:1 ratio

The homogenate is either filtered or centrifuged to recover the liquid phase

The solvent is washed with 1%NaCl solution , after vortexing one minute the mixture is centrifuged at low speed (2000 rpm) to separate the two phases

The lower chloroform phase containing lipid is separated.

4.2.5 REFLUX PROCESS :

The chloroform phase which have been separated is evaporated under vaccum concentrator

After evaporation, add 15 ml of methanolic NaOH and stir it for 15 mins then leave the solution to cool

Add 15ml of methanolic H_2SO_4 and stir it for 15 mins, leave the solution to cool

Transfer the solution in 50ml tube ,add heptane 1ml and water 2ml mix it well

Then separate the upper layer and inject in the gas chromatography

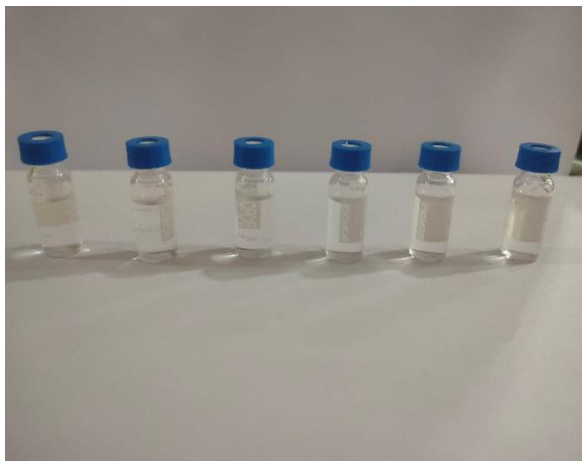


Fig.4 Sample for gas chromatography-mass spectrometry

4.2.6 GAS CHROMATOGRAPHY ANALYSIS

The injected sample is carried by a mobile phase (usually an inert gas such as helium or nitrogen) through a column containing a stationary phase (usually a polymer or a fused silica material coated with a thin layer of a stationary phase). The different components of the sample interact differently with the stationary phase, leading to their separation.

As the separated components elute from the column, they pass through a detector that measures the concentration of each component in the sample.

The output of the detector is recorded as a chromatogram, which is a plot of the detector response versus time. The peaks in the chromatogram correspond to the separated components in the sample, and the area under each peak is proportional to the concentration of that component.

RESULT AND DISCUSSION

5.1.FATTY ACID PROFILING – CONTROL

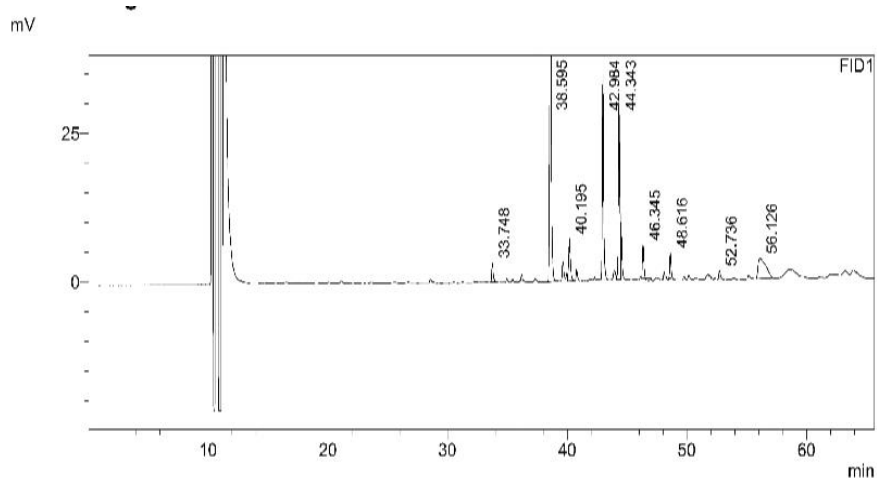


Table.2 Peak table for control

PEAK	RET.TIME	AREA	HEIGHT	NAME
1	33.78	29229	3248	Myristic Acid Methyl Ester
2	38.595	561604	64359	Palmitic Acid Methyl Ester
3	40.195	75493	7140	Palmitoleic Acid Methyl Ester
4	42.984	285023	34611	Stearic Acid Methyl Ester
5	44.343	264730	30453	Elaidic Acid Methyl Ester
6	46.345	37353	5541	Linoleic Acid Methyl Ester
7	48.616	34885	4317	Cis-11-Elcosenoic Acid Methyl Ester
8	52.736	12669	1366	Cis-11,14,17-Eicosatrienoic Acid Methyl Ester
9	56.126	151433	3389	Nervonic Acid Methyl Ester
TOTAL		1452419	154423	

Table 3:Percentage of fatty Acid present in control

S.NO	NAME	PERCENTAGE(%)
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1	Myristic Acid Methyl Ester	2.01
2	Palmitic Acid Methyl Ester	38.66
3	Palmitoleic Acid Methyl Ester	5.19
4	Stearic Acid Methyl Ester	19.62
5	Elaidic Acid Methyl Ester	18.22
6	Linoleic Acid Methyl Ester	2.57
7	Cis-11-Elcosenoic Acid Methyl Ester	2.40
8	Cis-11,14,17-Eicosatrienoic Acid Methyl Ester	0.87
9	Nervonic Acid Methyl Ester	10.42

FATTY ACID PROFILING-TREATED WITH 10 μ g/L FOR 24 HOURS

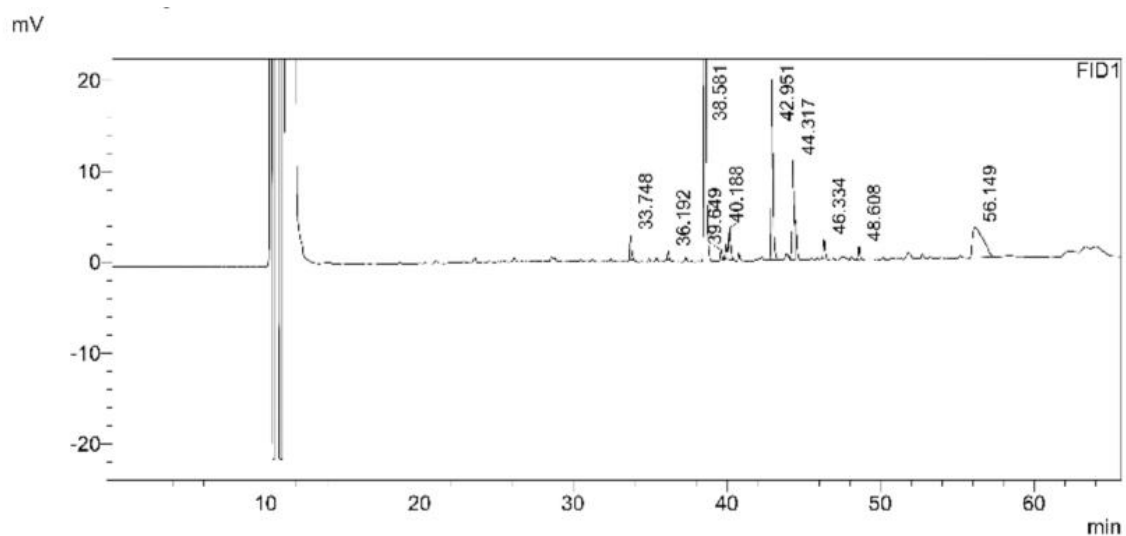


Table 4: Peak table for zebrafish testis which is treated with 10µg/L for 24 hrs

PEAK	RET.TIME	AREA	HEIGHT	NAME
1	33.748	21998	2691	Myristic Acid Methyl Ester
2	36.192	7057	941	Pentadecanoic Acid Methyl Ester
3	38.581	428958	48999	Cis-10-Pentadecanoic Acid Methyl Ester
4	39.649	7080	1017	Palmitoleic Acid Methyl Ester
5	40.188	44590	3503	Palmitoleic Acid Methyl Ester
6	42.951	164949	19771	Stearic Acid Methyl Ester
7	44.317	94456	10947	Elaidic Acid Methyl Ester
8	46.334	18116	2157	Linoleic Acid Methyl Ester
9	48.608	11236	1443	Cis-11-Elcosenoic Acid Methyl Ester
10	56.149	160716	3358	Nervonic Acid Methyl Ester
TOTAL		959156	94826	

FATTY ACID PROFILING-TREATED WITH 25µg/L FOR 24 HOURS

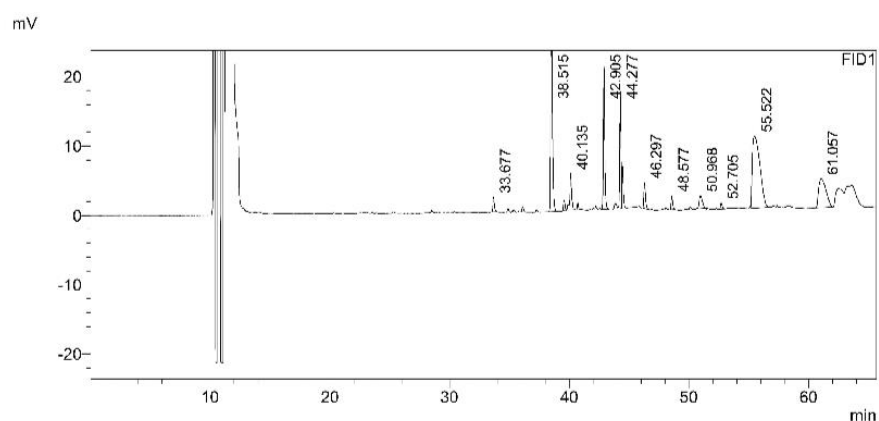


Table 5:Peak table for zebrafish testis which is treated with 25µg/L for 24 hrs

PEAK	RET.TIME	AREA	HEIGHT	NAME
1	33.677	18422	2120	Myristic Acid Methyl Ester
2	38.515	374919	43865	Cis-10- Pentadecanoic Acid Methyl Ester
3	40.135	66948	5245	Palmitoleic Acid Methyl Ester
4	42.905	167591	20568	Cis-10-Heptadecenoic Acid Methyl Ester
5	44.277	139743	16923	Elaidic Acid Methyl Ester
6	46.297	33725	3719	Linoleic Acid Methyl Ester
7	48.577	17201	2037	Cis-11-Elcosenoic Acid Methyl Ester
8	50.968	33109	1822	Behenic Acid Methyl Ester
9	52.705	8038	847	Cis-11,14,17-Eicosatrienoic Acid Methyl Ester
10	55.522	459558	10371	Cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester
11	61.057	177378	4152	Cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester
TOTAL		1496632	111669	

FATTY ACID PROFILING-TREATED WITH 50µg/L FOR 24 HOURS

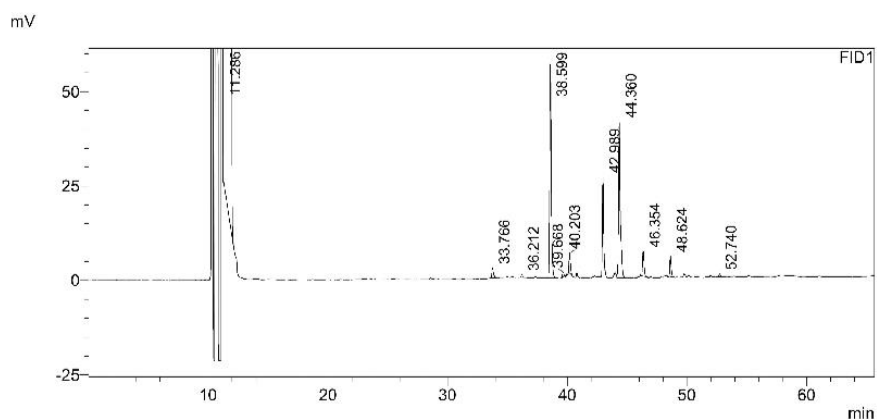


Table 6: Peak table for zebrafish testis which is treated with 50µg/L for 24 hrs

PEAK	RET.TIME	AREA	HEIGHT	NAME
1	11.286	7917220	397687	Butyric Acid Methyl Ester
2	33.766	26621	2649	Myristic Acid Methyl Ester
3	36.212	5637	715	Pentadecanoic Acid Methyl Ester
4	38.599	492657	56432	Palmitic Acid Methyl Ester
5	39.668	6890	1322	Palmitoleic Acid Methyl Ester
6	40.203	38919	5244	Palmitoleic Acid Methyl Ester
7	42.989	221547	26555	Stearic Acid Methyl Ester
8	44.360	417079	41303	Elaidic Acid Methyl Ester
9	46.354	52621	6544	Linoleic Acid Methyl Ester
10	48.624	42842	5430	Cis-11-Elcosenoic Acid Methyl Ester
11	52.740	7088	801	Cis-11,14,17-Eicosatrienoic Acid Methyl Ester
TOTAL		9229121	544682	

FATTY ACID PROFILING-TREATED WITH 100µg/L FOR 24 HOURS

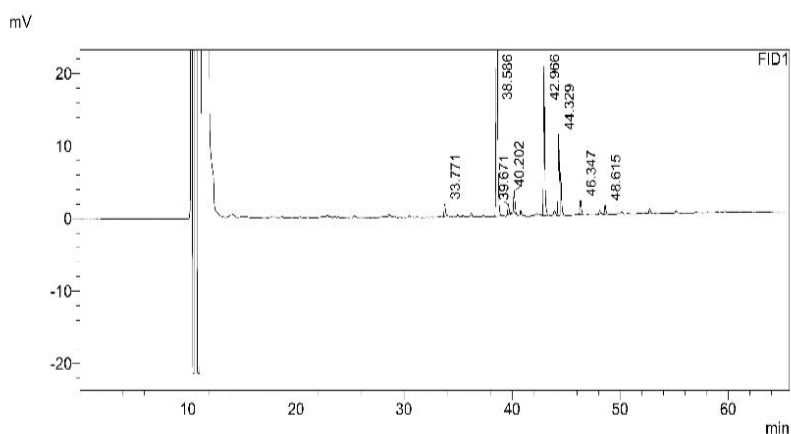


Table 7: Peak table for zebrafish testis which is treated with 100µg/L for 24 hrs

PEAK	RET.TIME	AREA	HEIGHT	NAME
1	33.771	15338	1726	Myristic Acid Methyl Ester
2	38.586	353830	40196	Cis-10- Pentadecanoic Acid Methyl Ester
3	39.671	8098	1325	Palmitoleic Acid Methyl Ester
4	40.202	23407	2905	Palmitoleic Acid Methyl Ester
5	42.966	172345	20473	Stearic Acid Methyl Ester
6	44.329	92697	11227	Elaidic Acid Methyl Ester
7	46.347	16518	1912	Linoleic Acid Methyl Ester
8	48.615	10864	1293	Cis-11-Elcosenoic Acid Methyl Ester
TOTAL		693098	81057	

FATTY ACID PROFILING-TREATED WITH 300µg/L FOR 24 HOURS

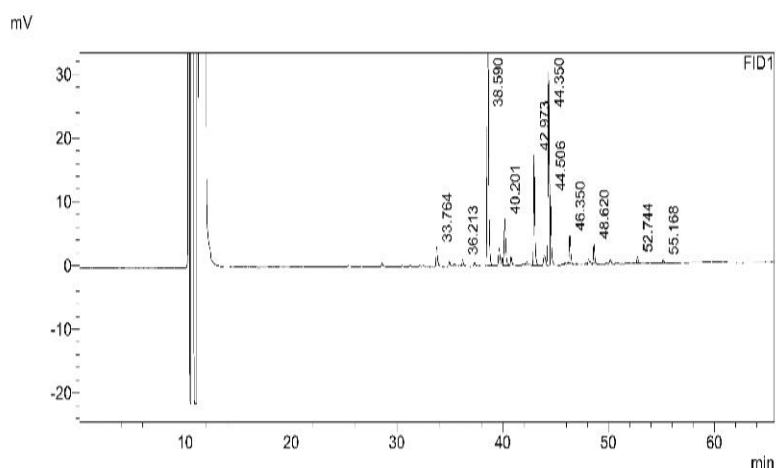


Table 8: Peak table for zebrafish testis which is treated with 300µg/L for 24 hrs

PEAK	RET.TIME	AREA	HEIGHT	NAME
1	33.764	27310	2969	Myristic Acid Methyl Ester
2	36.213	5999	854	Pentadecanoic Acid Methyl Ester
3	38.590	4022238	45453	Cis-10- Pentadecanoic Acid Methyl Ester
4	40.201	77884	7379	Palmitoleic Acid Methyl Ester
5	42.973	147630	17317	Stearic Acid Methyl Ester
6	44.350	262875	30417	Elaidic Acid Methyl Ester
7	44.506	86242	10963	Oleic Acid Methyl Ester
8	46.350	36827	4364	Linoleic Acid Methyl Ester
9	48.620	26795	3141	Cis-11-Elcosenoic Acid Methyl Ester
10	52.744	9653	1006	Cis-11,14,17-Eicosatrienoic Acid Methyl Ester
11	55.168	5439	512	Cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester
TOTAL		1088892	124375	

FATTY ACID PROFILING-TREATED WITH 10µg/L FOR 48 HOURS

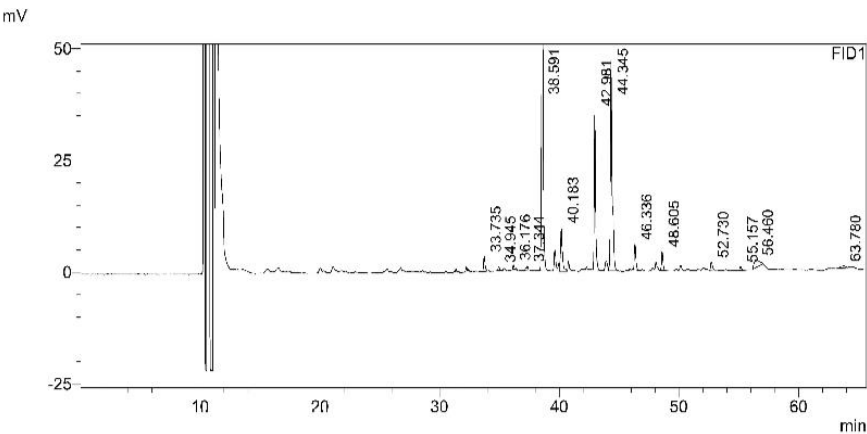


Table 9: Peak table for zebrafish testis which is treated with 10µg/L for 48 hrs

PEAK	RET.TIME	AREA	HEIGHT	NAME
1	33.735	31829	3381	Myristic Acid Methyl Ester
2	34.945	8177	890	Myristoleic Acid Methyl Ester
3	36.176	5087	947	Myristoleic Acid Methyl Ester
4	37.344	9212	878	Pentadecanoic Acid Methyl Ester
5	38.591	673513	76345	Cis-10- Pentadecanoic Acid Methyl Ester
6	40.183	108251	9766	Palmitoleic Acid Methyl Ester
7	42.981	295070	35674	Stearic Acid Methyl Ester
8	44.345	493343	46862	Elaidic Acid Methyl Ester
9	46.336	49173	5691	Linoleic Acid Methyl Ester
10	48.605	38059	4361	Cis-11-Eicosenoic Acid Methyl Ester
11	52.730	18747	1817	Cis-11,14,17-Eicosatrienoic Acid Methyl Ester
12	55.157	8227	766	Cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester
13	56.460	50781	1668	Nervonic Acid Methyl Ester
14	63.780	11361	474	Cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester
TOTAL		1800829	189520	

FATTY ACID PROFILING-TREATED WITH 25µg/L FOR 48 HOURS

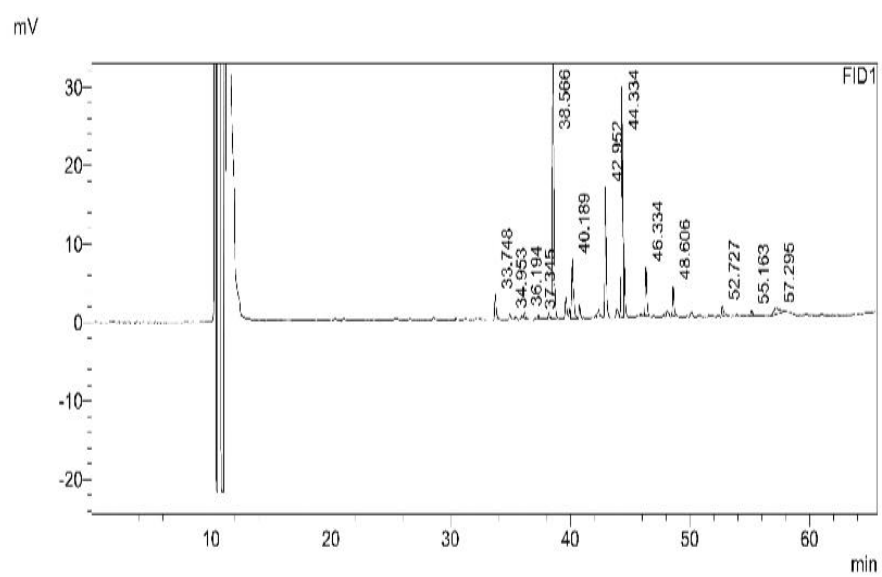


Table 10: Peak table for zebrafish testis which is treated with 25µg/L for 48 hrs

PEAK	RET.TIME	AREA	HEIGHT	NAME
1	33.748	28578	3169	Myristic Acid Methyl Ester
2	34.953	5915	705	Myristoleic Acid Methyl Ester
3	36.194	6382	827	Pentadecanoic Acid Methyl Ester
4	37.345	5291	526	Pentadecanoic Acid Methyl Ester
5	38.566	313963	36372	Cis-10- Pentadecanoic Acid Methyl Ester
6	40.189	84475	7655	Palmitoleic Acid Methyl Ester
7	42.952	140026	16654	Stearic Acid Methyl Ester
8	44.334	256924	29448	Elaidic Acid Methyl Ester
9	46.334	53297	6196	Linoleic Acid Methyl Ester
10	48.606	33157	3880	Cis-11-Elcosenoic Acid Methyl Ester
11	52.727	13413	1293	Cis-11,14,17-Eicosatrienoic Acid Methyl Ester
12	55.163	7204	711	Cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester
13	57.295	27956	689	Nervonic Acid Methyl Ester
TOTAL		976581	108126	

FATTY ACID PROFILING-TREATED WITH 50µg/L FOR 48 HOURS

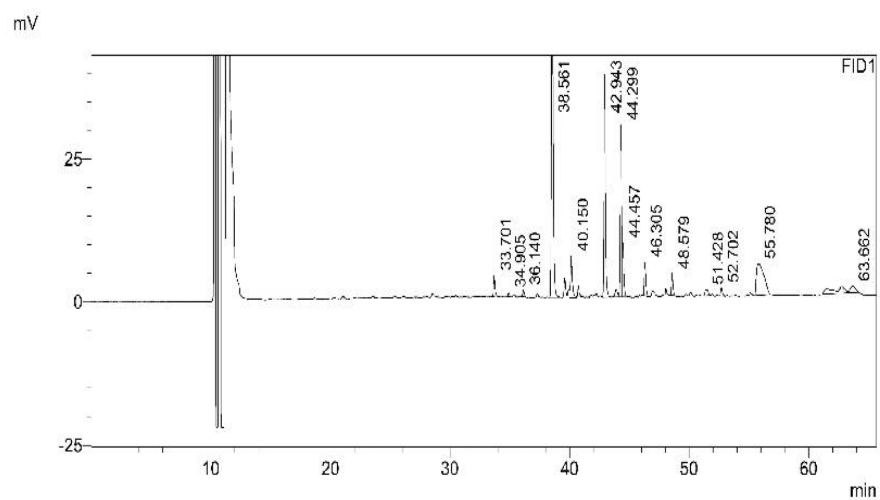


Table:11 Peak table for zebrafish testis which is treated with 50µg/L for 48 hrs

PEAK	RET.TIME	AREA	HEIGHT	NAME
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1	33.701	31216	3668	Myristic Acid Methyl Ester
2	34.905	5675	757	Myristic Acid Methyl Ester
3	36.140	11379	1224	Myristoleic Acid Methyl Ester
4	38.561	692173	78812	Cis-10- Pentadecanoic Acid Methyl Ester
5	40.150	80080	7290	Palmitoleic Acid Methyl Ester
6	42.943	328916	38813	Stearic Acid Methyl Ester
7	44.457	255220	30072	Elaidic Acid Methyl Ester
8	46.305	70760	9486	Oleic Acid Methyl Ester
9	48.579	50495	5873	Linoleic Acid Methyl Ester
10	48.579	38058	3837	Cis-11-Elcosenoic Acid Methyl Ester
11	51.428	20309	1082	Behenic Acid Methyl Ester
12	52.702	14072	1469	Cis-11,14,17-Eicosatrienoic Acid Methyl Ester
13	55.780	243033	5587	Nervonic Acid Methyl Ester
14	63.662	2114297	1117	Cis-4,7,10,13,16,19- Docosahexaenoic Acid Methyl Ester
TOTAL		1955582	189087	

FATTY ACID PROFILING-TREATED WITH 100µg/L FOR 48 HOURS

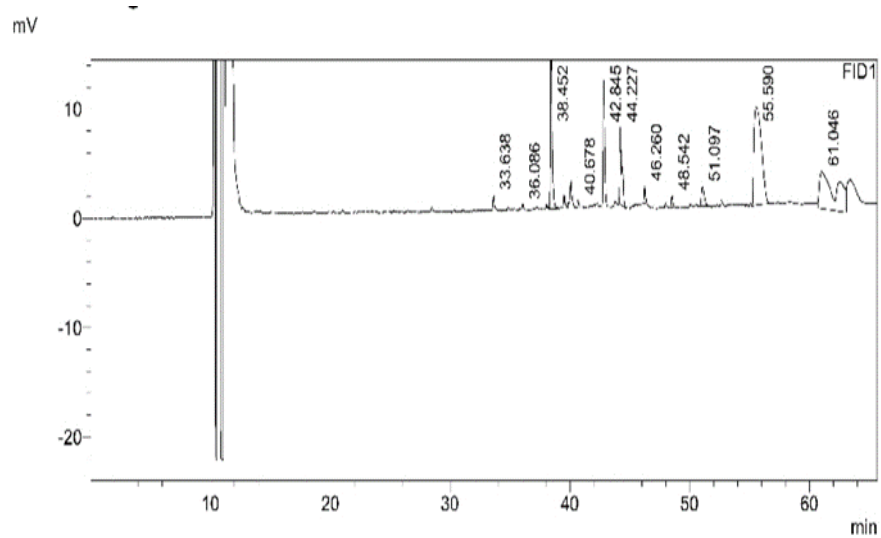


Table: 12 Peak table for zebrafish testis which is treated with 100µg/L for 48 hrs

PEAK	RET.TIME	AREA	HEIGHT	NAME
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1	33.638	11333	1342	Myristic Acid Methyl Ester
2	36.086	3555	460	Myristoleic Acid Methyl Ester
3	38.452	195524	22116	Cis-10- Pentadecanoic Acid Methyl Ester
4	40.678	5198	663	Palmitoleic Acid Methyl Ester
5	42.845	94847	11515	Cis-10-Heptadecenoic Acid Methyl Ester
6	44.227	86442	7083	Elaidic Acid Methyl Ester
7	46.260	30544	1598	Linoleic Acid Methyl Ester
8	48.542	8184	1042	Cis-11-Elcosenoic Acid Methyl Ester
9	51.097	12653	1761	Behenic Acid Methyl Ester
10	55.590	391797	8988	Cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester
11	61.046	323245	3411	Cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester
TOTAL		1163323	59979	

FATTY ACID PROFILING-TREATED WITH 300µg/L FOR 48 HOURS

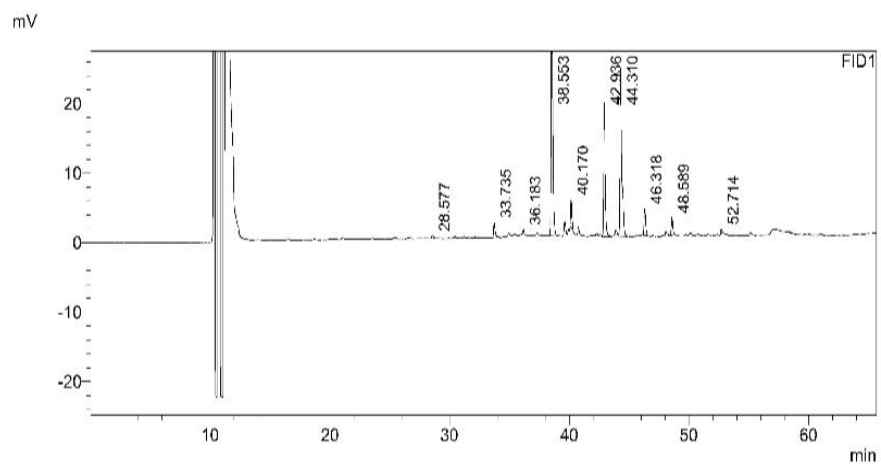


Table:13 Peak table for zebrafish testis which is treated with 300µg/L for 48 hrs

PEAK	RET.TIME	AREA	HEIGHT	NAME
1	28.577	3391	348	Lauric Acid Methyl Ester

2	33.735	19199	2144	Myristic Acid Methyl Ester
3	36.183	6237	755	Myristoleic Acid Methyl Ester
4	38.553	344679	39554	Cis-10- Pentadecanoic Acid Methyl Ester
5	40.170	30287	4030	Palmitoleic Acid Methyl Ester
6	42.936	165731	19210	Cis-10-Heptadecenoic Acid Methyl Ester
7	44.310	255068	24026	Elaidic Acid Methyl Ester
8	46.318	33753	3863	Linoleic Acid Methyl Ester
9	48.589	21915	2566	Cis-11-Elcosenoic Acid Methyl Ester
10	52.714	4342	638	Cis-11,14,17- Eicosatrienoic Acid Methyl Ester
TOTAL		884601	97133	

FATTY ACID PROFILING-TREATED WITH 10µg/L FOR 72 HOURS

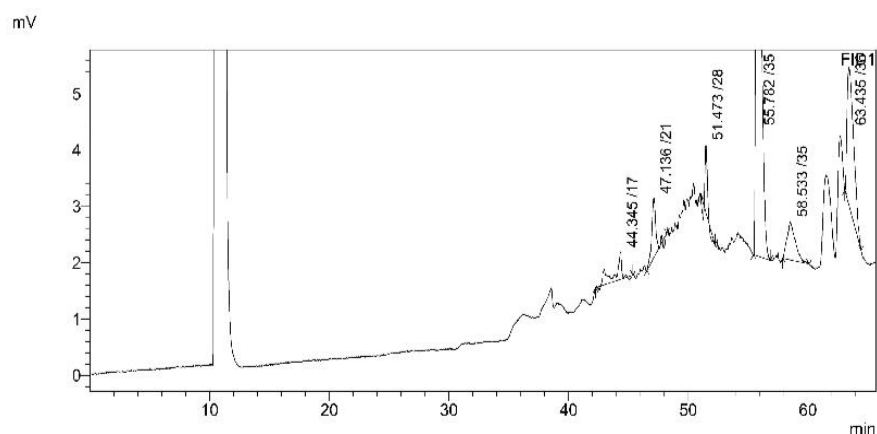


Table:14 Peak table for zebrafish testis which is treated with 10µg/L for 72 hrs

PEAK	RET.TIME	AREA	HEIGHT	NAME
1	44.345	16655	488	Eladic Acid Methyl Ester
2	47.136	22280	1051	Arachidic Acid Methyl Ester
3	51.473	10750	1207	Cis-8,11,14,-Eicosatrienoic Acid Methyl Ester
4	55.782	249514	7455	Nervonic Acid Methyl Ester
5	58.533	32017	685	Nervonic Acid Methyl Ester
6	63.435	95255	2456	Cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester
TOTAL		426470	13341	

FATTY ACID PROFILING-TREATED WITH 25µg/L FOR 72 HOURS

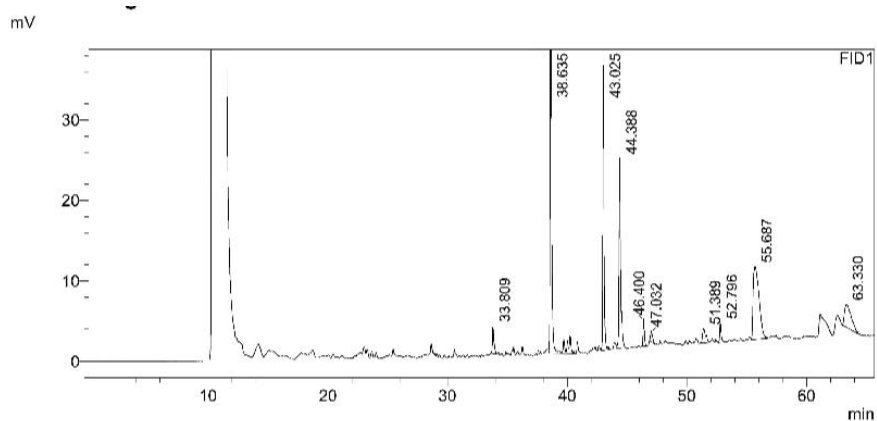


Table:15 Peak table for zebrafish testis which is treated with 25µg/L for 72 hrs

PEAK	RET.TIME	AREA	HEIGHT	NAME
1	33.809	30366	3384	Myristic Acid Methyl Ester
2	38.635	474762	62318	Palmitic Acid Methyl Ester
3	43.025	253095	35345	Stearic Acid Methyl Ester
4	44.388	177582	23671	Oleic Acid Methyl Ester
5	46.400	112684	3354	Linoleic Acid Methyl Ester
6	47.032	14878	1315	Arachidic Acid Methyl Ester
7	51.389	32394	1755	Behenic Acid Methyl Ester
8	52.796	21718	2421	Tricosanoic Acid Methyl Ester
9	55.687	309431	9030	Cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester
10	63.330	23887	2859	Cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester
TOTAL		1450796	145453	

FATTY ACID PROFILING-TREATED WITH 50µg/L FOR 72 HOURS

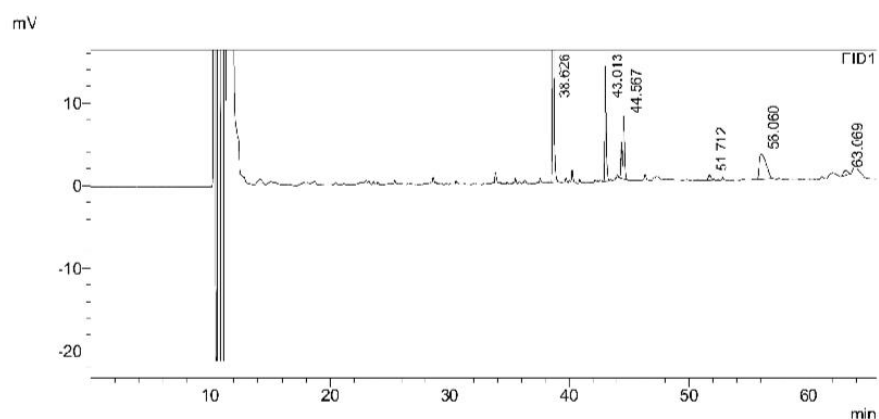


Table16: Peak table for zebrafish testis which is treated with 50µg/L for 72 hrs

PEAK	RET.TIME	AREA	HEIGHT	NAME
1	38.626	225269	29108	Palmitic Acid Methyl Ester
2	43.013	102850	13902	Stearic Acid Methyl Ester
3	44.567	89109	7511	Oleic Acid Methyl Ester
4	51.712	9810	605	Cis-8,11,14,-Eicosatrienoic Acid Methyl Ester
5	56.060	115032	3106	Nervonic Acid Methyl Ester
6	63.069	135032	544	Cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester
TOTAL		555522	54775	

FATTY ACID PROFILING-TREATED WITH 100µg/L FOR 72 HOURS

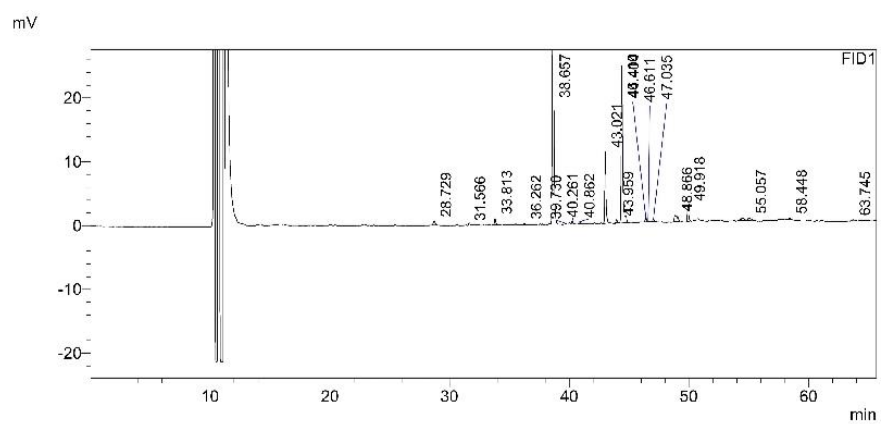


Table:17 Peak table for zebrafish testis which is treated with 100µg/L for 72 hrs

PEAK	RET.TIME	AREA	HEIGHT	NAME
1	28.729	7655	659	Lauric Acid Methyl Ester
2	31.566	6	-4	Tridecanoic Acid Methyl Ester
3	33.813	7971	1013	Myristic Acid Methyl Ester

4	36.262	1892	244	Pentadecanoic Acid Methyl Ester
5	38.657	410083	53763	Palmitic Acid Methyl Ester
6	39.730	1106	142	Palmitoleic Acid Methyl Ester
7	40.261	3419	415	Palmitoleic Acid Methyl Ester
8	40.862	1707	318	Heptadecanoic Acid Methyl Ester
9	43.959	3349	442	Elaidic Acid Methyl Ester
10	44.404	5524	24553	Oleic Acid Methyl Ester
11	46.410	199362	709	Linoleic Acid Methyl Ester
12	46.611	-244	0	Linoleic Acid Methyl Ester
13	47.035	3461	454	Arachidic Acid Methyl Ester
14	48.866	18082	971	A-Linoleic Acid Methyl Ester
15	49.918	18928	2551	Cis-11,14,-Eicosatrienoic Acid Methyl Ester
16	55.057	18152	463	Lignoceric Acid Methyl Ester
17	58.488	2614	233	Nervonic Acid Methyl Ester
18	63.745	2786	194	Cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester
TOTAL		787243	98365	

FATTY ACID PROFILING-TREATED WITH 300µg/L FOR 72 HOURS

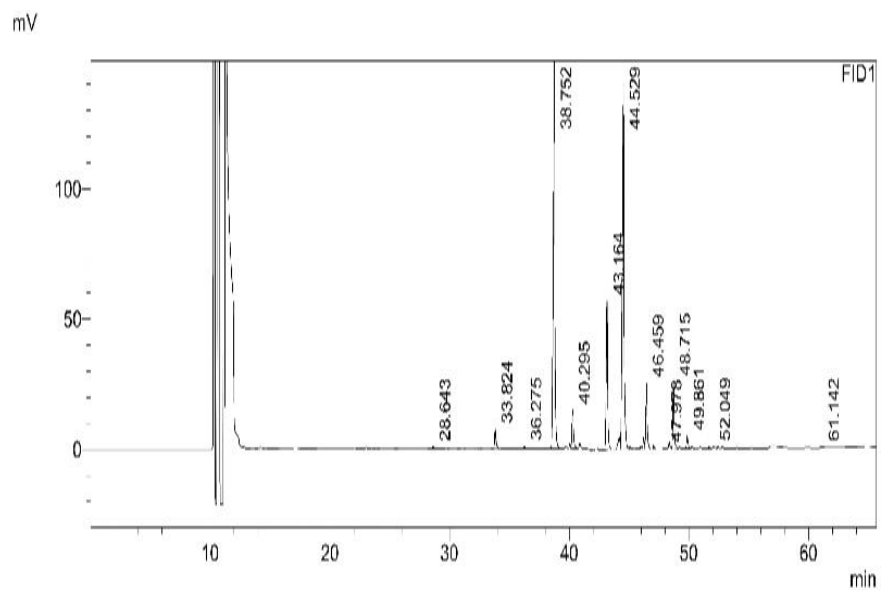


Table:17 Peak table for zebrafish testis which is treated with 300µg/L for 72 hrs

PEAK	RET.TIME	AREA	HEIGHT	NAME
1	28.643	4781	560	Lauric Acid Methyl Ester

2	33.824	58769	7227	Myristic Acid Methyl Ester
3	36.275	6530	920	Pentadecanoic Acid Methyl Ester
4	38.752	1390470	150109	Palmitic Acid Methyl Ester
5	40.295	127320	14765	Palmitoleic Acid Methyl Ester
6	43.164	459512	57149	Stearic Acid Methyl Ester
7	44.529	1392117	139817	Oleic Acid Methyl Ester
8	46.459	213786	25334	Linoleic Acid Methyl Ester
9	47.978	3474	393	G-Linoleic Acid Methyl Ester
10	48.715	211525	25760	A- Linoleic Acid Methyl Ester
11	49.861	38365	4758	Cis-11,14-Eicosadienoic Acid Methyl Ester
12	52.049	6338	462	Cis-8,11,14,-Eicosatrienoic Acid Methyl Ester
13	61.142	3879	357	Cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester
TOTAL		3916867	427612	

Table:18 Percentage of fatty acid present in 10µg/L

S.NO	NAME	24 hrs	48 hrs	72 hrs
1	Myristic Acid Methyl Ester	2.29	1.76	—
2	Pentadecanoic Acid Methyl Ester	0.73	0.51	—
3	Cis-10-Pentadecanoic Acid Methyl Ester	44.7	37.40	—
4	Palmitoleic Acid Methyl Ester	4.64	6.01	—
5	Stearic Acid Methyl Ester	17.1	16.38	—
6	Elaidic Acid Methyl Ester	9.84	27.39	—
7	Linoleic Acid Methyl Ester	1.88	2.73	—
8	Cis-11-Elcosenoic Acid Methyl Ester	1.17	2.11	
9	Nervonic Acid Methyl Ester	16.7	2.81	58.50
10	Myristoleic Acid Methyl Ester	—	0.45	—
11	Cis-11,14,17-Eicosatrienoic Acid Methyl Ester	—	1.04	2.52
12	Cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester	—	0.45	—
13	Cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester	—	0.63	22.33
14	Elaidic Acid Methyl Ester	—	—	3.90
15	Arachidic Acid Methyl Ester	—	—	5.22
S.NO	NAME	24 hrs	48 hrs	72 hrs
1	Myristic Acid Methyl Ester	2.29	1.76	—
2	Pentadecanoic Acid Methyl Ester	0.73	0.51	—
3	Cis-10-Pentadecanoic Acid Methyl Ester	44.7	37.40	—
4	Palmitoleic Acid Methyl Ester	4.64	6.01	—
5	Stearic Acid Methyl Ester	17.1	16.38	—
6	Elaidic Acid Methyl Ester	9.84	27.39	—
7	Linoleic Acid Methyl Ester	1.88	2.73	—
8	Cis-11-Elcosenoic Acid Methyl Ester	1.17	2.11	
9	Nervonic Acid Methyl Ester	16.7	2.81	58.50
10	Myristoleic Acid Methyl Ester	—	0.45	—
11	Cis-11,14,17-Eicosatrienoic Acid Methyl Ester	—	1.04	2.52
12	Cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester	—	0.45	—

13	Cis4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester	—	0.63	22.33
14	Eladic Acid Methyl Ester	—	—	3.90
15	Arachidic Acid Methyl Ester	—	—	5.22

Table:19 Percentage of fatty acid present in 25µg/L

S.NO	NAME	24 hrs	48 hrs	72hrs
1	Myristic Acid Methyl Ester	1.23	2.92	2.09
2	Cis-10- Pentadecanoic Acid Methyl Ester	25.05	32.14	—
3	Palmitoleic Acid Methyl Ester	4.47	8.65	—
4	Cis-10-Heptadecenoic Acid Methyl Ester	11.19	—	—
5	Elaidic Acid Methyl Ester	9.33	26.30	—
6	Linoleic Acid Methyl Ester	2.25	5.45	7.76
7	Cis-11-Elcosenoic Acid Methyl Ester	1.14	3.39	—
8	Behenic Acid Methyl Ester	2.21	—	2.23
9	Cis-11,14,17-Eicosatrienoic Acid Methyl Ester	0.53	1.37	—
10	Cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester	30.70	0.73	21.32
11	Cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester	11.85	—	1.64
12	Myristoleic Acid Methyl Ester	—	0.60	—
13	Pentadecanoic Acid Methyl Ester	—	0.65	—
14	Palmitic Acid Methyl Ester	—	—	33.72
15	Stearic Acid Methyl Ester	—	—	17.44
16	Oleic Acid Methyl Ester	—	—	12.24
17	Arachidic Acid Methyl Ester	—	—	1.02
18	Tricosanoic Acid Methyl Ester	—	—	1.49

Table:20 Percentage of fatty acid present in 50µg/L

s.no	NAME	24 hrs	48 hrs	72 hrs
1	Butyric Acid Methyl Ester	85.78	—	—
2	Myristic Acid Methyl Ester	0.28	1.59	—
3	Pentadecanoic Acid Methyl Ester	0.06	—	—
4	Palmitic Acid Methyl Ester	5.33	—	40.55
5	Palmitoleic Acid Methyl Ester	0.42	4.09	—
6	Stearic Acid Methyl Ester	22.40	16.81	18.51
8	Elaidic Acid Methyl Ester	4.51	13.05	—
9	Myristoleic Acid Methyl Ester	—	0.58	—
10	Cis-10- Pentadecanoic Acid Methyl Ester	—	35.39	—
11	Oleic Acid Methyl Ester	—	3.61	16.04
12	Linoleic Acid Methyl Ester	—	2.58	—
13	Cis-11-Elcosenoic Acid Methyl Ester	—	1.94	—
14	Behenic Acid Methyl Ester	—	1.03	—
15	Cis-11,14,17- Eicosatrienoic Acid Methyl Ester	—	0.71	1.76
16	Nervonic Acid Methyl Ester	—	12.42	20.70
17	Cis-4,7,10,13,16,19- Docosaheaxanoic Acid Methyl Ester	—	5.84	2.42

Table:21Percentage of fatty acid present in 100µg/L

S.NO	NAME	24 hrs	48 hrs	72 hrs
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1	Myristic Acid Methyl Ester	2.21	0.97	0.012
2	Cis-10- Pentadecanoic Acid Methyl Ester	51.05	16.80	–
3	Palmitoleic Acid Methyl Ester	3.37	0.44	0.43
4	Stearic Acid Methyl Ester	24.86	–	11.73
5	Elaidic Acid Methyl Ester	13.37	7.43	0.42
6	Linoleic Acid Methyl Ester	2.38	2.62	25.32
7	Cis-11-Elcosenoic Acid Methyl Ester	1.56	0.70	–
8	Myristoleic Acid Methyl Ester	–	0.30	–
9	Cis-10-Heptadecenoic Acid Methyl Ester	–	8.15	–
10	Behenic Acid Methyl Ester	–	1.08	–
11	Cis-5,8,11,14,17- Eicosapentaenoic Acid Methyl Ester	–	33.67	–
12	Cis-4,7,10,13,16,19- Docosaheaxenoic Acid Methyl Ester	–	27.78	0.35
13	Lauric Acid Methyl Ester	–	–	9.7
14	Tridecanoic Acid Methyl Ester	–	–	0.0007
15	Pentadecanoic Acid Methyl Ester	–	–	0.24
16	Palmitic Acid Methyl Ester	–	–	52.09
17	Heptadecanoic Acid Methyl Ester	–	–	0.21
18	Oleic Acid Methyl Ester	–	–	0.70
19	Arachidic Acid Methyl Ester	–	–	0.43
20	A- Linoleic Acid Methyl Ester	–	–	2.29
21	Cis-11,14,- Eicosatrienoic Acid Methyl Ester	–	–	2.40
22	Lignoceric Acid Methyl Ester	–	–	2.30
23	Nervonic Acid Methyl Ester	–	–	0.33

Table:22 Percentage of fatty acid present in 300µg/L

S.NO	NAME	24 hrs	48hrs	72hrs
1	Myristic Acid Methyl Ester	2.50	2.17	1.50
2	Pentadecanoic Acid Methyl Ester	0.55	—	0.16
3	Cis-10- Pentadecanoic Acid Methyl Ester	36.94	38.96	—
4	Palmitoleic Acid Methyl Ester	7.15	3.42	3.25
5	Stearic Acid Methyl Ester	13.55	—	11.73
6	Elaidic Acid Methyl Ester	24.14	28.83	—
7	Oleic Acid Methyl Ester	7.92	—	35.54
8	Linoleic Acid Methyl Ester	3.38	—	5.45
9	Cis-11-Eicosenoic Acid Methyl Ester	2.46	—	—
10	Cis-11,14,17-Eicosatrienoic Acid Methyl Ester	0.88	—	0.16
11	Cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester	0.49	—	—
12	Lauric Acid Methyl Ester	—	0.38	0.122
13	Myristoleic Acid Methyl Ester	—	0.70	—
14	Cis-10-Heptadecenoic Acid Methyl Ester	—	18.73	—
15	Palmitic Acid Methyl Ester	—	—	35.49
16	A-Linoleic Acid Methyl Ester	—	—	5.40
17	G-Linoleic Acid Methyl Ester	—	—	0.08
18	Cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester	—	—	0.09

DISCUSSION:

Fatty acid profiling is a technique used to analyze the composition of different fatty acids in a biological sample. In this case, the fatty acid profiling of zebrafish testis treated with fipronil can provide valuable information about the impact of this

pesticide on the lipid metabolism of the fish. Fipronil is a widely used insecticide that has been shown to have toxic effects on fish, including zebrafish. The testis is an important organ for reproduction and any disturbance in its function can have significant effects on the population of the fish. Li et al. (2016) investigated the impact of fipronil exposure on the fatty acid profile of male zebrafish liver and found that exposure to this pesticide led to significant alterations in the composition of several fatty acids, including a decrease in the level of unsaturated fatty acids. Liao et al. (2018), the authors investigated the effects of fipronil exposure on the lipid metabolism of zebrafish and found that exposure to this pesticide led to significant changes in the expression of genes involved in fatty acid synthesis and oxidation. These changes were accompanied by alterations in the fatty acid composition of the fish tissues. Fatty acids play a crucial role in various physiological processes, including membrane structure and function, energy metabolism, and cell signaling, and changes in their composition can have significant effects on the health and reproductive fitness of organisms. Fipronil is known to act as a disruptor of the endocrine system in animals, and its effects on fish reproduction have been documented in previous studies. The fatty acid profiling of zebrafish testis treated with fipronil can provide insights into the potential mechanisms underlying these effects, such as alterations in the synthesis and metabolism of sex hormones. Fipronil exposure can have significant effects on the fatty acid profile of zebrafish tissues, including the testis.

CHAPTER-6

SUMMARY AND CONCLUSION:

Fipronil is an insecticide commonly used in agriculture and can be toxic to fish and other aquatic organisms. Studies have shown that exposure to fipronil can lead to changes in fatty acid profiles in fish, potentially affecting their growth, reproduction, and overall health. Fatty acid profiling is a useful tool for understanding the

physiological effects of toxicants such as fipronil on fish. By analyzing the fatty acid composition of fish tissues, researchers can identify changes in lipid metabolism and determine the potential mechanisms of toxicity. An insecticide commonly used in agriculture, on the fatty acid composition of zebrafish testis. In this study I found that exposure to fipronil led to significant changes in the fatty acid profile of the testis, including a decrease in Stearic acid and an increase in linoleic acid. These changes in the fatty acid profile were concentration-dependent, with higher concentrations of fipronil leading to more significant effects. The findings suggest that fipronil exposure can disrupt lipid metabolism in zebrafish testis, potentially affecting their reproductive health.

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