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Research Article

BIOACCUMULATION OF ORGANOPHOSPHATE PESTICIDE IN ORGANS OF SNAKE HEAD FISH AND THEIR EFFECT ON BIOCHEMICAL MOIETIES

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ABSTRACT

Fishes are very sensitive to aquatic environmental changes so they are important bio-indicator for monitoring the contamination of water bodies. Numerous organophosphate pesticides are widely used in domestic, industrial and agricultural fields to protect the crops from various pest and diseases owing to their high insecticidal property and low persistence in the environment. Pesticide exposure may also fatal to many non-target organisms like fish where it hampers its health through impairment of metabolism. Dichlorvos is an organophosphate insecticide and widely used in all fields. Present study was aimed to evaluate accumulation of pesticide in tissues and the effects of pesticide residues on biochemical moieties in fish, *Channa gachua* exposed to organophosphate pesticide dichlorvos. In present study, fishes were exposed to 1/10thof LC50 concentration (12.5 ppm) of dichlorvos for 30 days (chronic). Highly significant depletion was observed in glycogen, protein and lipid content in liver and muscle of fishes exposed to pesticide as compared to control. Accumulation of pesticide residue was also observed in those tissues by HPLC method. Biotransformation and bioaccumulation of pesticide and their residues in food chain can cause detrimental health effects on non-target organism.

Keywords: Organophosphate pesticides, Biochemical moieties, Bioaccumulation, Chronic.

1. INTRODUCTION

With the increasing population, industrialization and revolution in science and technology, human beings are continuously disturbing the delicate environmental balance in ecosystems. Toxic chemicals are deliberately introduced into the environment including pesticides, heavy metals and effluents. Now adays use of pesticides is tremendously increased to control pests in agricultural, industrial and domestic fields. In early decades organophosphorus pesticides were employed in medicine and industry, because of their relatively low persistence due to biodegradability. Due to unprecedented growth in human population, pesticides have now become a necessity of farmers to increase crop yield and fulfill the requirement of sufficient amount of food. But, when chemicals, fertilizers and pesticides are applied in the field, they affect on survival, growth, metabolism and reproduction of non-target organisms.

The fish is a good indicator and highly sensitive in such ecosystem where the water gets contaminated to toxic chemicals. Dichlorvos belongs to widely used organophosphorus group of pesticides. It is hazardous chemical and has been known to accumulate in fish tissue and other edible organisms thereby having a chance to reach the predators like birds and man through food chain. Extensive biochemical studies have been carried out on the effect of pesticide on fishes. Sing *et al*. evaluated effect of cypermethrin on biochemical profile of the fish, *C. fasciatus* [1], similarly Tantarpale in tissues of *C. striatus* exposed to cypermethrin [2], Vasantharaja *et al*. in fish, *C. mrigala* exposed to cypermethrin [3], Magar and Dube in *C. punctatus* exposed malathion [4], Shaikh and Gautam in *H. fossilis* exposed to nuvan [5], Justin and Josef in *O. mossambicus* exposed to acetamipride [6]. Alterations in biochemical parameters like protein and carbohydrates indicated the susceptibility of organ to pollutant followed by altering their function. The biochemical changes in fish tissues used as a biological indicator for pollution with pesticides. So the present study was aimed to evaluate alterations in biochemical constituents and bioaccumulation of pesticide in liver and muscle of fresh water edible fish, *C. gachua* after exposure to potent insecticide dichlorvos.

2. MATERIAL AND METHOD

2.1. Selection of fish

In present research work the fresh water fish, *Channa gachua* was selected keeping in mind its availability throughout the year in local river Krishna around Karad city, Dist. Satara.

2.2. Selection of pesticide

In present study organophosphate pesticides *i.e.* dichlorvos was used for toxicity testing against the selected fish, which were purchased from local agro chemist shop.

2.3. Research Design and Methodology

Fishes were divided in to two groups *viz.* i) Group I (control group), ii) Group II treated with $1/10^{th}$ (1.25 ppm) of LC_{50}.

2.3.1. Chronic toxicity

For the chronic toxicity study $1/10^{th}$ (1.25 ppm) LC_{50} concentrations (12.5 ppm) of pesticide was selected. The chronic toxicity experiment were conducted for 30 days. After 30 days alive fishes were sacrificed and desired organs were quickly excised to study the various parameters by employing the methods.

2.4. Biochemical Parameters estimation

Anthrone method was used to estimate total glycogen content in respective tissues [7]. Total protein content was estimated with Folin Ciocalteau reagent by Lowry's method [8]. Total lipid content was estimated with Sulphophosphovaniline reagent by Bernes and Black-stock method [9]. The results of biochemical estimations were analyzed by one way ANOVA method.

2.5. HPLC Analysis [10]

2.5.1. Tissues extraction

Liver and muscle of the fishes were exposed to LC_{50} concentration of pesticide. The tissue (1 gm) was first minced with scissors and then homogenized in homogenizer with acetonitrile (5 ml). The homogenate was filtered through filter paper (Whatman No. 1) and the remnants of tissue were re-homogenised and re-extracted twice with acetonitrile (5×2 ml) and filtered through filter paper. The acetonitrile filtrates were transferred to a clean, dry separator funnel and n-hexane was added to it (1:1). The mixture was shaken vigorously for 2 min and allowed to settle (2 min) till the two phases were distinctly separated. The lower acetonitrile phase was collected in another separator funnel and re-partitioned twice with n-hexane. The acetonitrile phase was then collected in another conical flask and evaporated to dryness using rotary vacuum evaporator. Final volume was made to 1 ml with acetonitrile (HPLC grade). The sample was filtered through 0.20µ membrane filter before injection to HPLC.

Mobile phase: Acetonitrile and Water (90:10). Column: 5µ Luna C18 (2); 250 × 4.6 mm (RP). Wave length: 254 nm, Retention time: 20 min. Standard and sample (20 µl each) were injected by Hamilton Syringe into the injector port of liquid chromatography with the first and last being the standard. The concentration of dichlorvos in tissue was calculated using the following equation:

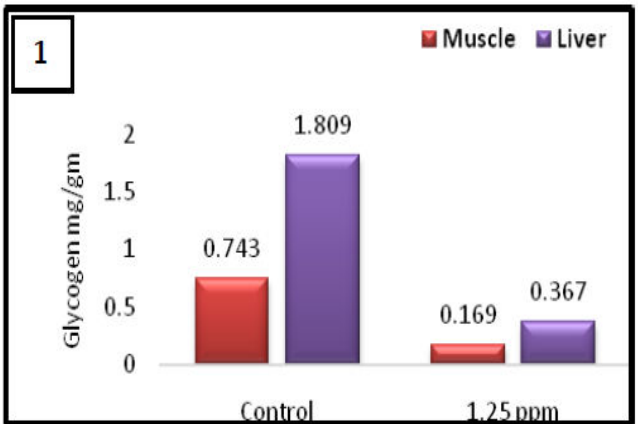
Residue of dichlorvos in tissues (µg/gm) = a2 × v2 × C/ a1× g

a1=area of standard chromatogram, a2=area of sample chromatogram

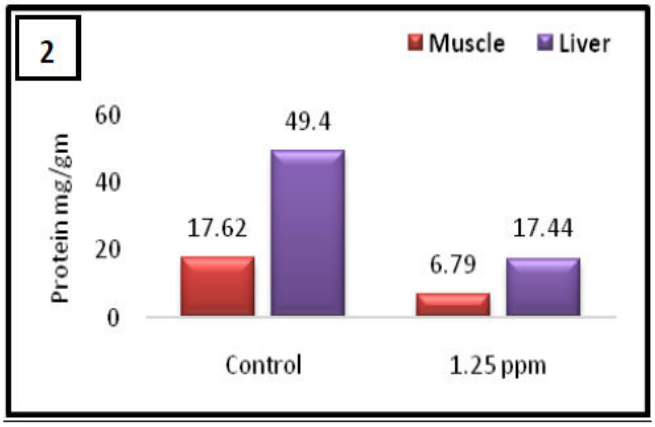
V2=Final volume of sample after processing (ml), V1=Volume of sample taken for processing (ml), C = concentration of standard, g = amount of tissue taken for processing.

3. RESULT AND DISCUSSION

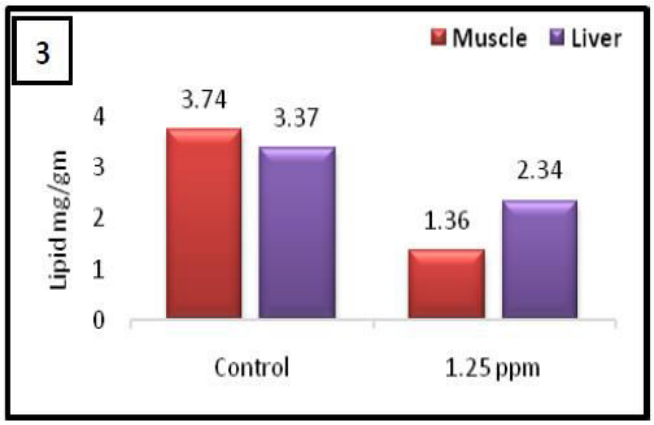
In the present research work, the amount of glycogen, protein and lipid in some important organs like muscle and liver was estimated in control fish and in fishes were exposed to different concentrations of dichlorvos. Exposure period and concentration of pesticide dependent alterations were observed in the levels of these biochemical constituents in the organs under investigations over control fish which are illustrated graphically by graphs 1-3.



Graph 1: Changes in glycogen content in muscle and liver of *C. gachua* to 1.25 ppm of dichlorvos after chronic exposure



Graph 2: Changes in protein content in muscle and liver of *C. gachua* to 1.25 ppm of dichlorvos after chronic exposure



Graph 3: Changes in lipid content in muscle and liver of *C. gachua* to 1.25 ppm of dichlorvos after chronic exposure.

Biochemical constituents are source of energy in animal body which is present in the form of stored energy and used in starved and stressed condition. When fishes get exposed to polluted water, the physiological functioning of organs system get disturbed that induces toxic effect at cellular and molecular level, which leads into biochemical alterations [11]. The pesticides induce alterations in normal functioning of cells with subsequent alterations in biochemical mechanism in fish [12]. According to Verma and Tonk, fluctuation in the biochemical constituents indicate the susceptibility of organ system to toxicants [13].

3.1. Glycogen

The estimated values of the glycogen were 1.8097 mg in liver and 0.7438 mg in muscle per gm. wet weight of each tissue in control fish. In fishes exposed to 1.25 ppm (1/10th of LC_{50}) concentration, the percent depletion was more in liver (79.71%) followed by muscle (77.36%) as compared to control (graph 1). Glycogen is the prime and important biochemical constituents in tissues of fish. It acts as building blocks and reservoir of chemical energy in the cell which can increase or decrease according to organismal need [14]. Due to pesticidal stress, such prime and important energy source is affected significantly and alters various processes in the fish. Decrease in the glycogen content of various organs of fish, exposed to pesticides, have been reported earlier by number of workers. Reduced glycogen content was reported in muscle, gill, liver and kidney of fish, *A. dussumieri* after dimethoate treatment by Rathode *et al.* [15]. Similar results have also been reported by Ganeshwade *et al.* in various organs such as muscle and liver of fish, *P. ticto* due to dimethoate toxicity stress [16]. Significant decline in glycogen content was observed in liver and muscle of fish, *L. rohita* after exposed to cypermethrin [17]. Similar results have also been observed by Veeraiah *et al.* in muscle and liver of same fish after sublethal exposure of indoxacarb [18], by Neeraja and Giridhar in liver and muscle of *L. rohita* exposed to deltamethrin [19], by Nirmala *et al.* in gill, brain, kidney, liver and muscle of fish, *L. rohita* exposed to pyrachlostrobin [20], and by Hussain *et al.* in blood serum of fish, *C. catla* and *L. rohita* exposed to dimethoate [21].

The results in present investigation showed significant decrease in the glycogen level in muscle and liver of fish, *C. gachua* exposed to dichlorvos at chronic exposure period. These results were in agreement and correlate with the finding of earlier workers. Here, decrease in glycogen was might be due to the rapid utilization of stored glycogen for energy production in pesticide stressed condition at acute exposure. Required Energy in the form of ATP might be produced due to anaerobic breakdown of glycogen which may leads to breakdown of more amount of glycogen to cope up the energy need under stress condition. During stress organisms need sufficient energy which is supplied from reserved material *i.e.* glycogen. Kabeer suggested that the decrease in glycogen content in malathion treated fresh water fish, *T. mossambica* may be due to decrease in glycogen synthesis [22]. Significant decrease of glycogen might be because of these organs become active during stress condition and they may require large amount of energy. This energy demand might be solved by utilizing reserved food material in the form of glycogen resulting in to its decrease. Less intake of glucose might

be due to pesticide contaminated food material and rapid utilization of glycogen might results into the reduction in glycogen content in tissues of pesticide induced fish. Depletion in the tissue glycogen content may depend upon the intensity of the pesticide to disturb the tissue and capacity of the tissue to get ride off [23]. After entry of pesticide in the organ system of fish the metabolites of pesticide might interfere with the metabolic process in the tissue and affect enzyme activities essential in the glycogenesis and it may result into inhibition of such cycle collapse or stop the conversion of glucose in to glycogen.

3.2. Protein

The estimated values of the protein were 49.409 mg in liver and 17.624 mg in muscle per gram wet weight of each tissue in control fish. In fishes exposed to 1.25 ppm concentration of this pesticide, protein depletion was highly significant ($p<0.001$) in both organs. The percent depletion was more in liver (64.98%), followed by muscle (61.45%) at this concentration (graph 2). Protein serves as an alternative source of energy for animal when the insufficient energy is available from the other sources like carbohydrates and lipids. Protein plays an important role in cellular metabolism and regulates intra and extra cellular interactions media as a part of cell membrane and enzyme [24]. In present study, protein showed a highly significant decrease in both tissues of fishes exposed to $1/10^{th}$ of LC 50 concentration (chronic) of pesticide. The decrease in protein content has been reported by some workers in numerous fishes exposed to various toxicants. Tripathi and Verma found endosulfan mediated reduction in protein content of liver and muscle in fish, *C. batrachus* [25]. Sing indicated a significant decrease in total protein content in muscle and liver of fish, *C. fasciatus* exposed to sublethal concentrations of cypermethrin [26]. According to him, the depletion in protein in various tissues due to its degradation and utilization of degraded products for metabolic purposes. Vidhya and Nair also showed decreasing trend in protein content in liver and muscle of fish, *Etroplussuratensis* exposed to different concentration of lambadacyhalothrin [27]. Nagraju and Venkata Rathnamma stated decrease in protein content of tissue indicated the physiological adaptability of the fish to compensate pesticide stress [28]. To overcome the stress the animals require high energy. This energy demand might be led to protein catabolism. Similar protein depletion was also observed by Chamarthi*et al.* in quinalphos induced fish, *C. carpio* up to 15 days [29], and by Asomba and Ugokwe in fish, *Clarias albopunctatus* [30]. Tamizhazhagan *et al.* found decrease in protein content in muscle and liver of fish, *C. catla* after different exposure period of monochrotophos [31]. The results obtained in the present investigation are in accordance with observations of earlier workers. Here it is assumed that the significant reduction in protein level was might be due to the pesticidal stress. As mentioned earlier protein acts as an alternative source of energy in fishes exposed to certain toxicants and it plays important role in cellular metabolism. To overcome pesticidal stress fish demands high energy and this energy demand might have to stimulate proteolysis. On the other hand the proteins might be utilized for the gluconeogenesis pathway for the synthesis of glucose for energy production and there might be increased degradation of amino acids. This might fed to TCA cycle through amino transferase for energy production as stated earlier by Rajput *et al.*[32]. Protein might be used in cell repair, tissue organization and formation of lipoproteins, which are important cellular constituents of cell membranes and cell organelles present in the cytoplasm [33]. Decrease in protein in liver may be due to the effect of toxic compounds and their interference with hepatocellular protein synthesis. These toxic compounds inhibit the incorporation of amino acids into proteins and increased degradation of protein into amino acids [23].

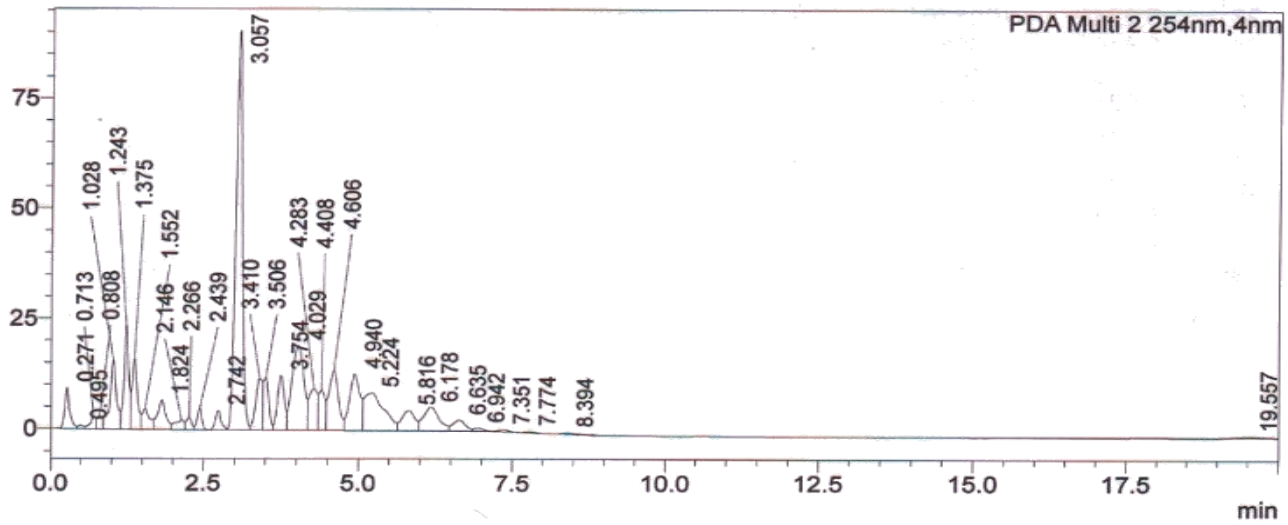
3.3. Lipid

The estimated values of the lipid was 3.747 mg in muscle and 3.379 mg in liver per gram wet weight of each tissue in control fish.The percent depletion was highly significant ($p<0.001$) at 1.25 ppm concentration. In muscle depletion of lipid content was more (63.65%). The liver also showed highly significant (30.57%) depletion in lipid but less than other tissues. There was highly significant decrease in lipid content in both the tissues of fishes exposed to 1.25 ppm of dichlorvos when compared to control (graph 3). Lipid is an essential biochemical constituent in tissues of all animals, and plays vital role in the energy metabolism [14]. Lipids are the best energy producers in the body other than carbohydrate. Due to toxic stress, such important biochemical compound is affected and fluctuated. In the present study the lipid content was decreased in both tissues except liver of fish, chronic exposure to dichlorvos. Similar results were reported by Kamble [23]. He observed decrease in the lipid content of liver and muscle of chronically exposed fish,

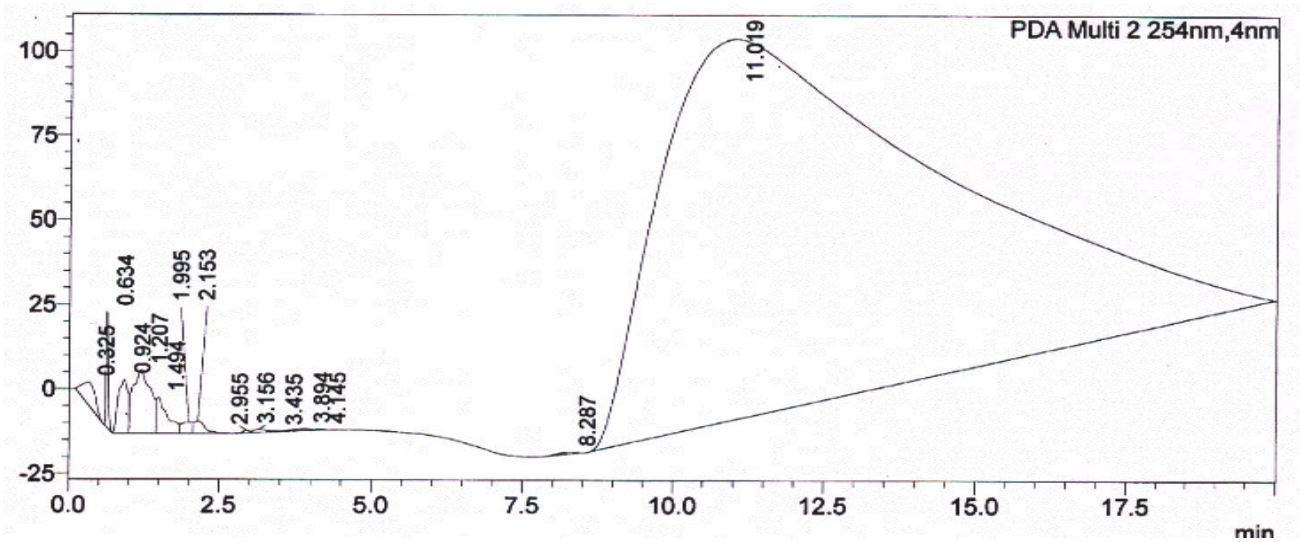
S. mossambicus to endosulfan and chlorpyriphos. Tazeen *et al.* mentioned that the reduction in lipid level is due to excessive lipolysis and subsequently used for synthesis of glucose to meet the high energy demand by the fish [34]. According to Binukumari and Vasanthidecrease in lipid content in the tissues of *O. mossambicus* exposed to chlorpyriphos indicates its enhanced hydrolysis to derive energy to overcome pesticidal stress [35]. Bhavan and Geraldine also support this phenomenon and stated that under the pesticide stress condition the hydrolysis of lipid may be accelerated to cope up with the high energy demand [36]. Padma Priya and AvsanMaruthi showed significant decrease in lipid content in liver tissue of fish, *C. punctatus* after exposure of different concentration of imidaclopride for 96 hrs [37]. Lipid content decreased in liver and muscle of *L. rohita* exposed to sodium fluoride (NaF) in sub-lethal and lethal concentration [38]. According to them decreased lipid level might be due to inhibition of lipid synthesis by sodium fluoride as well as increased utilization of stored lipid as a source of energy to conduct regular metabolic functions. Pechiammal and Kiruthika observed similar decreasing trend in gill, liver, muscle and kidney of fish, *C. mrigala* exposed to rogor [39]. Tamizhazhagan *et al.* supports earlier results [31]. Results obtained in present study are in agreement with earlier workers. Here it is assumed that the depletion in lipid level might be due to the lipolysis in different tissues to overcome the high energy demand by the fish under pesticidal stress. Pesticides may exerts their toxic effect on metabolism of fish and fish may needs extra energy to cope up with pesticidal stress, so the rate of lipolysis might be accelerated for the production of energy and subsequently used for glucose synthesis. On the other hand it is suggested that the lipid synthesis may be inhibited and mobilization of stored lipids through β-oxidation and unsaturation of lipid molecules may occurred [40]. In long term exposure the lipids are the main and important source of energy in the fish so at chronic exposure under pesticidal stress the lipid might be decreased. Pesticide mayaffects on cellular structure of the tissue and lipid might be used in cell repair and tissue organization due to that depletion in lipid content may occur.

3.4. HPLC Analysis

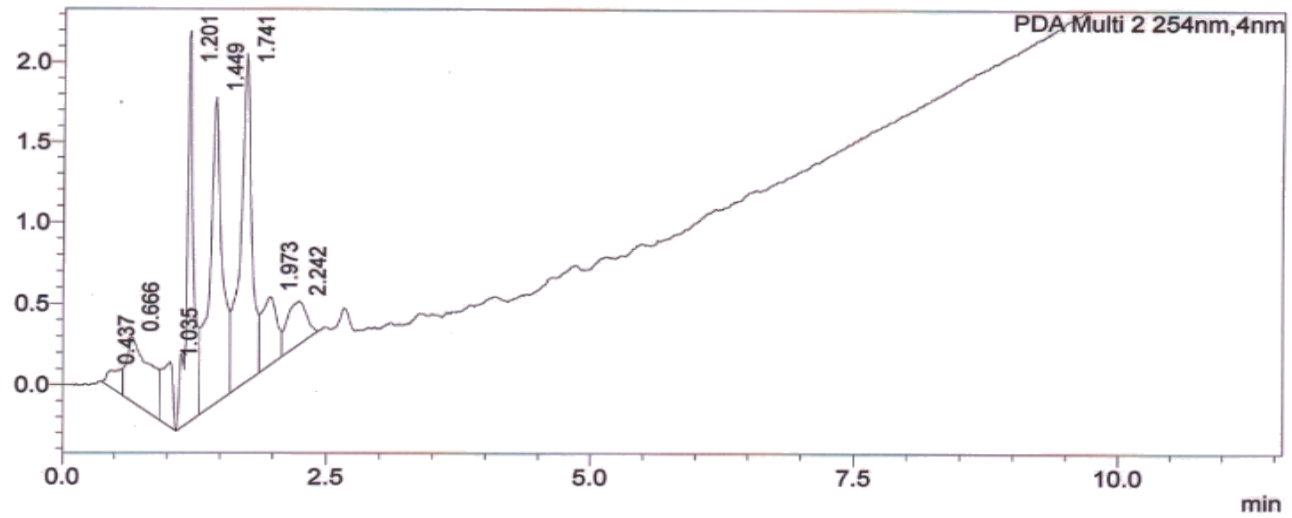
Presence of dichlorvos in liver and muscle in fishes exposed to $1/10^{th}$ of LC_{50} concentration for 30 days was measured by HPLC. The chromatogram (graph 4, 5 and 6) showed a well resolved peak of dichlorvos and the retention time was at 20 minutes under the operating condition of the separation and quantification of dichlorvos by HPLC is better at wavelength of 254 nm with mobile phase of acetonitrile and water (90:10 v/v). The concentration of dichlorvos in tissue was calculated using the above (mention in method) formula. In present investigation the concentration of dichlorvos in muscle was 29.83 µg/gm. of weight of tissue. The conc. of dichlorvos in liver was 0.84 µg/gm. of weight of tissue.From earlier research it is understood that dichlorvos was stated to be producing biochemical, metabolic and physiological in different tissues of fish but we cannot conclusively say like that without any proof.



Graph 4: Typical Chromatogram of standard dichlorvos



Graph 5: Typical Chromatogram of Muscle in fishes exposed to dichlorvos.



Graph 6: Typical Chromatogram of Liver in fishes exposed to dichlorvos.

Dichlorvos residue in water refers to its concentration that has remained in the water after it has been added to water bodies. The presence of dichlorvos residue in water after 30 days similar observation was made by Manjunatha*et al.*, they stated that the amount of chlorpyrifos added to water was 200µg/L and it was remained in water up to the 96 hrs. but the concentration of chlorpyrifos was decreased when time period was increased [41]. This phenomenon was supported by Saad *et al.*, according to them the organophosphorus compounds are quickly degradable in the aquatic environment [42]. However these compounds accumulate in the tissues and cells of the fishes and exerted their effects on the biochemistry and physiology of them. The residue of the pesticide found in the liver and muscle of the present fish studied. Muscle get intimate contact with toxic medium and might be the primary rout for the uptake of water pollutants. However the liver serves as a storage organs for vast variety of nutrient. Accumulation of this pesticide in the muscle and liver can also be as a result of detoxification mechanisms and may originate from pesticides deposited in the aquatic environment. However, the liver is the preferred organs for pesticides accumulation as could be deduced from the present study. Accumulation of pesticides is the function of their respective membrane permeability and enzyme system, which is highly species specific and because of this fact pesticides accumulated in tissues of the fish [43].

4. CONCLUSION

The widespread use of pesticides in agriculture raise toxicological and environmental problems resulting potential toxic effects in human and animals. The pesticide intoxication might disturb the normal functioning of the cell with the alterations in the biochemical mechanism in fish. This may results in the mortality of fish on chronic or acute exposure to the pesticide. The pesticide might be deposited in fish accidentally or by means of contaminated water bodies with pesticide and it might lead to harmful effects in human being on continuous consumption of those fish.

5. ACKNOWLEDGEMENT

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