**Original Article**

**Evaluation of Bioconcentration of Organophosphate Pesticides Monocrotophos and Quinalphos in Freshwater Fish Channa striatus**

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**INTRODUCTION**

Contemporary agriculture depends heavily on synthetic chemicals, which include different types of fertilizers, pesticides, and herbicides, or weedicides. Pesticide application is widely practiced in rice and rice-based cropping systems. Most of these delicate ecosystems are seriously affected due to the indiscriminate use of pesticides. Pesticides and weedicides not only alter the physicochemical characteristics of aquatic systems but also affect the aquatic organisms as a whole (De Vlaming et al. 2000, Dener, 2000). Because of their toxic nature, there is a general concern with the potential hazards of pesticides to humans and the environment (Braunbeck, 1994b). The excess quantities of pesticides produce unwanted and unwarranted residues, which pose a great threat to aquatic organisms (Thurupp, 1991). The natural mechanisms like photolysis, chemical decomposition, and microbial decomposition are suitable mechanisms of nature itself to detoxify these chemicals. But due to many reasons, in most of the aquatic ecosystem, the functioning of the natural mechanisms to eliminate and detoxify poisons was found to be deficient. This may lead to longer pesticide persistence in water. The manifestation of pesticide residues in an aquatic ecosystem raises an imperative question regarding the potentials of these residues to enter and concentrate in the aquatic organisms. Two major hazards of pesticides residues in water are the disposition of chemicals in the bodies of the fishes and in the bodies of aquatic organisms that forms the food for fishes and as a result of both, more chemical gets deposited in the fish than is found in water (Howard, 2017). Pesticide intake takes place mainly in three ways: dermally (direct absorption through skin by swimming in contaminated water), direct uptake through the gills during respiration and orally by drinking pesticide-contaminated water or feeding on contaminated prey.

The bioaccumulation of an insecticide depends on its persistence in the ecosystem, i.e. availability which leads to bioconcentration and biomagnification. Bioconcentration is the accumulation of pesticides in animal tissue at levels greater than in water to which they are applied. Hence bioconcentration of pesticides in the various elements of the aquatic food chain is a...
A perusal of the available literature has revealed that most of the studies were concentrated on organochlorine pesticides and hence organophosphate pesticides, carbamates, and pyrethroid pesticides received less attention regarding their bioconcentration changes. In a way, the comparatively high probabilities of organochlorine pesticides to undergo biomagnification at various trophic levels encouraged workers to go deep in this line and as a result, the other branches of pesticides were left behind. Some of the OPs and pyrethroids that are applied directly to the surface water to control mosquitos and other vectors enhance the chances of surface water contamination with greater chances of bioaccumulation. The present study is an attempt to look into the possibilities of bioconcentration in fish Channa striatus under laboratory conditions using selected pesticides from organophosphates which are commonly used in Kerala.

MATERIALS AND METHODS

The fish selected for the study were Channa striatus which is commonly found in the paddy fields, ponds, tributaries, and rivers of Kerala. Moreover, C. striatus has a place in the menu of fishermen. C. striatus for the study were collected with the help of local fishermen from an isolated pond to avoid any possible contamination. Uninjured healthy fishes of almost similar size and weight were chosen from the lot and transferred to the laboratory in a closed water-filled bucket and acclimatized to the ambient laboratory temperature of 28±0.20°C in a large cement tank. 25% freshwater was added once every two days after removing the same amount of water from the tank. The fishes were fed with rice flour mixed with a fish meal in the ratio 1:1. After seven days of acclimatization 20 healthy fishes were captured from the stock tank and 10 each was introduced into the two experimental tanks. The tanks were rectangular measuring a length of 90cm and a depth of 60cm. The tanks were filled with 100 litres of freshwater collected from a nearby well. Tank A was considered as experimental and tank B as control. Fishes were starved for two days before the actual experiment. The temperature, pH, and DO of the water introduced were noted at intervals (temp -28±1°C, pH - 6.5 ± 0.5, and DO - 7 ± 0.5mg/l).

Organophosphates namely Monocrotophos and Ekalux were selected for the study. Sublethal doses of Monocrotophos and Ekalux for C. striatus were adopted from Laly (1988). Fishes kept in cement tank were exposed to sublethal doses of monocrotophos (0.01ppm/l) and ekalux (0.00025ppm/l) separately for 21 days. Separate experiments were conducted for individual insecticides. The values obtained in μ/l in bioassay studies were converted to ppm/l for convenience and unification while presenting and discussing the results. Commercial grade pesticides namely Phoskill (Monocrotophos 36%), manufactured by United Phosphorous Ltd, Gujarat, Ekalux (Quinalphos 25% EC) manufactured by Segentia India Ltd, Mumbai. Fishes in the control tank were maintained properly without any contamination.

Fishes were fed with rice flour and fish meal in the ratio 1:1 once daily and the tanks were thoroughly monitored throughout the study. Dead fishes were removed immediately and precautions were taken to see minimum mortalities in the tanks. 25 liters of water from each tank were removed once in two days and care was taken to maintain the same concentration of pesticides in the medium.

After the experiment period of 21 days, fishes were captured from both tanks, sacrificed, and dissected. Tissues such as gills, liver, and flesh were removed, cleaned, and kept in separate containers, and transported to the laboratory under chilled condition (-20°C). The analysis was performed with Perkin-model 5890 Gas Chromatography equipped with Ni 63 electron capture detector following the official methods of AOAC 2007. 01 (AOAC, 201).

Bioconcentration factors (BCFs) of pesticides in aquatic plants were determined by the following expression:

\[ BCF = \frac{C_B}{C_W} \]

Where Cb and Cw respectively represent the concentration of pesticides in biota (aquatic plants) and water.

RESULTS AND DISCUSSIONS

Bioconcentration studies of the organophosphate pesticide monocrotophos in various tissues of C. striatus viz. gills, liver, and muscle are depicted in Table 1. Irrespective of the nature of the sample tissues, all the samples were detected at ppm concentration. Monocrotophos residues concentrated maximum in gills (0.033 mg/kg), followed by liver (0.016mg/kg) and muscles (0.012) (Table 1 & Fig 1). The bioconcentration factor values obtained were 3.3, 1.6, and 1.2 for gills, liver, and muscle respectively (Table 2 & Fig 2). Quinalphos concentrated more in the liver (0.98mg/kg) and then in gills (0.210mg/kg) and least in the muscle (0.074) (Table 3 & Fig 3) and the bioconcentration factor values obtained were 840, 3920, and 296 in gills liver and muscles respectively (Table 4 & Fig 4). These results were obtained when the fishes were introduced to sub lethal doses of 0.01ppm/l and 0.00025ppm/l, (Laly, 1988), of monocrotophos and quinalphos respectively.
**Table 1:** Bio concentration of Monocrotophos in various tissues of *C. striatus* (0.01ppm/l in the medium)

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Experimental (mg/kg)</th>
<th>Control</th>
<th>Detection Limit (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gills</td>
<td>0.033</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>Liver</td>
<td>0.016</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>Muscles</td>
<td>0.012</td>
<td>0</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Fig 1** Showing bio concentration of monocrotophos in *Channa striatus*

**Table 2:** Bio concentration factors of Monocrotophos in various tissues of *Channa striatus*

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Experimental (mg/kg)</th>
<th>Concentration of exposure (ppm/l)</th>
<th>Bio concentration facts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gills</td>
<td>0.033</td>
<td>0.01ppm</td>
<td>3.3</td>
</tr>
<tr>
<td>Liver</td>
<td>0.016</td>
<td>0.01ppm</td>
<td>1.6</td>
</tr>
<tr>
<td>Muscles</td>
<td>0.012</td>
<td>0.01ppm</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*Bio concentration factor = accumulated concentration in tissue (mg/kg) / concentration in the media (ppm)*

**Fig 2** Showing bioconcentration factor of monocrotophos
Table 3: Bioconcentration of quinalphos in various tissues of *C. striatus* (0.00025ppm/l in the experimental medium)

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Experimental (mg/kg)</th>
<th>Control</th>
<th>Detection Limit (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gills</td>
<td>0.210</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>Liver</td>
<td>0.98</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>Muscles</td>
<td>0.074</td>
<td>0</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Fig 3 Showing bio concentration of quinalphos in *Channa striatus*

Table 4: Bio concentration factors of quinalphos in various tissues of *Channa striatus*

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Experimental (mg/kg)</th>
<th>Concentration of exposure (ppm/l)</th>
<th>Bio concentration factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gills</td>
<td>0.210</td>
<td>0.00025ppm</td>
<td>840</td>
</tr>
<tr>
<td>Liver</td>
<td>0.98</td>
<td>0.00025ppm</td>
<td>3920</td>
</tr>
<tr>
<td>Muscles</td>
<td>0.074</td>
<td>0.00025ppm</td>
<td>296</td>
</tr>
</tbody>
</table>

Fig 4 Showing bioconcentration factor of Quinalphos
Xenobiotic compounds are fat-soluble, readily taken up from the water, sediments, and food sources into the tissues of aquatic organisms (Walker and Livingstone, 1992). According to Haitzer et al. (1998), hydrophobic xenobiotic compounds bioaccumulate and bioconcentrate on aquatic organisms.

The four pesticides tested in the present study fall into two major categories of pesticides employed in the Kuttanad region, namely organophosphates and pyrethroids. Organochlorine pesticides are widely substituted by organophosphate pesticides and pyrethroid pesticides in recent years. Organophosphate pesticides act on the neurotransmitter enzyme acetylcholinesterase (AChE) in insects, humans, and many other animals (Costa 2008). Organophosphate toxicity of different species varies with age, weight, and sex of animals (Gill et al. 1988). Their residues have been detected in groundwater, drinking water, natural surface waters, marine organisms, and food products (Barcelo et al. 1990, Barcelo, 1993, Gunderson, 1995, Dejonkeheer et al.1996, Hernandez et al. 1996). OP insecticides contaminate the aquatic environment through agricultural runoff (Young and Nicholson, 1951). OP insecticides are regarded as pollutants due to their potential toxicity, mutagenicity, and carcinogenicity. Though they have a shorter half-life in tropical conditions their extensive and repeated application often leads to build-up residues in the environment (Saha, 2017). Pyrethroids are relatively stable for a longer duration (Walia, 2017). The potential toxicity of a pesticide is due to its extreme hydrophobicity and lipophilic nature (Coast, 1990). All pyrethroids are not equally soluble in water.

Among OPs, monocrotophos is a major pesticide applied in Kuttanad which is soluble in water and slightly soluble in mineral oil. Its half-life period in neutral water is 66 days. According to FAO and WHO, joint meeting on pesticide residue (1993) the acceptable daily intake (ADI) was 0 to 0.00006 mg/kg. In the present study monocrotophos accumulated in gills (0.033mg/kg), liver (0.016mg/kg and muscles (0.012). All these values were beyond the acceptable limit for human consumption as set by FAO/WHO. Quinalphos is another extensively used OP insecticide in Indian agriculture Babu et al. 1992, Armes et al, 1992). Quinalphos is known to be susceptible to hydrolysis. It undergoes photodegradation on exposure to natural sunlight with a half-life of 30 days in distilled water (Dureja et al. 1988). Reports of quinalphos residue are scanty in fishes and other vertebrates. The present study suggests that prolonged exposure to quinalphos can lead to high residue accumulation in tissues of fishes. This observation is in agreement with the observations of Bradbury et al. (1987), Tripathi, (1992), Tilak et al. (2003) and (2004). In the present study, the 21 day experimental set up revealed the concentration of quinalphos in the gills (0.210mg/kg), liver (0.98mg/kg) and muscles (0.074mg/kg) of C. striatus. The ADI set for quinalphos by the joint meeting on pesticide residue in 1984 and 1984 was 0.002mg/kg. All values in the present study were slightly above the ADI limits. The accumulation of residues followed a pattern in which the liver stood first followed by gills and muscles. A similar result was observed by (Rajgure, 2014) may due to the nature of particular insecticide which needs further investigations. The very low sub-lethal dose of ekalux (0.00025ppm/l) was potent enough to accumulate the pesticide residues in the various organs of fish C. striatus in the present study. Ekalux showed more potential to bio concentrate in a lower sub-lethal concentration than monocrotophos in C. striatus since the sub-lethal dose of monocrotophos was 0.01 ppm/l.

The presence of toxins in the edible portions of the fish is a matter of serious concern. Though not been able to magnify, the pesticides in the edible portions can cause immediate health problems to consumers. Organophosphate pesticides, monocrotophos, and ekalux used in the present study showed accumulation tendency in the various organs tested. Monocrotophos concentrated 0.012mg/kg and quinalphos 0.074mg/kg in muscles. The doses applied in the present study were 0.00025ppm/l (sub-lethal concentration) of ekalux and 0.1ppm/l monocrotophos. A comparison of the above aspects revealed the highly toxic and bioconcentration nature of quinalphos. Monocrotophos is banned by the Government of Kerala but quinalphos is not banned and is available in the market and extensively used.

In fishes, gills rapidly absorb pesticide residues from freshwater (Lloyd, 1992) and tend to bioconcentrate much fold over the residue in water (Devi et al. 1981). The liver is the major organ for the metabolism of the residues in fish (Paterson and Bately, 1993). In the present study among the four pesticides tested, ekalux accumulated more in the liver than gills and muscles. Various studies showed that endosulfan and carbofuran residues also tend to accumulate more in liver than other internal organs like the intestine, gills, brain and skeletal muscles (Dureja 2012, Berbert et al. 1989, Ferrando et al. 1992, Singh and Garg, 1992, Kale et al. 1996). Du Preez et al. (1997) found high levels of heavy metal residues in gills and liver of fish Clarias gariepinus collected from
Olifants River, South Africa. In the present investigation monocrotophos, lambda-cyhalothrin and fenvalerate followed a different pattern where gills exhibited a maximum value followed by liver and muscle respectively. The lipophilic nature of pyrethroids enables gills to absorb these pesticides quickly (Rehman et al. 2014).

The nature of the pesticide and the type of fish determine the ability of the pesticide to concentrate on various organs of the animal tested. According to Li. (2017) the distribution of a given amount of pyrethroids between water and sediment is governed by an equilibrium partitioning coefficient based on the amount of organic carbon available into the system. Sediment organic carbon plays a major role in determining the bioavailability of a given pyrethroid pesticide in aquatic systems and the pyrethroid toxic potential. Sediments may act as a source of pesticides to the overlying waters through the exchange at the sediment-water interface (Farmer et al. 1995). In the aquatic systems, hydrophobic chemical pollutants rapidly associate with particles in water and are deposited in sediments thus consumed by benthic organisms (Mc Elroy et al. 1989). C. striatus, being a bottom preferring fish has more chances for pesticide entry in the various organs. Biotransformation by fish appears to limit bioaccumulation. The present study indicates a significant reduction in the amount of pesticide residue concentrated in the flesh which may be due to biotransformation. Toxicity profiles observed during prolonged, constant exposure in the laboratory may not accurately reflect toxicological responses (Barron and Woodburn, 1995). But studies like this are pointers regarding the possibilities of these pesticides to enter the food chain if applied indiscriminately. Toxic substances incorporated in the food chain at lower trophic levels may result in harmful effects to organisms at higher trophic levels including man. According to Hoffman (2002), pesticides may act on fishes by indirectly affecting food chains. Sub lethal concentrations of various pesticides in water and food may accumulate in fishes especially in paddy fields and ponds. Fishes in paddy fields may not be able to escape the pesticides due to the embankments around and are forced to remain in the field. This may increase the possibilities of bioaccumulation in paddy fields than rivers and streams.

In Kerala the majority of people depend on fish and fish products for their daily nutrient requirements. The poor consume low-value fishes like Anabas scandans, Etroplus maculatus, C. striatus, etc. and sell out high-value fishes like E. suratensis, Valigo attu, etc.

CONCLUSION

The rivers and lakes are surrounded by hectares of agricultural areas especially paddy fields. The pesticides applied to the crops may enter the rivers by leaching and may cause long term exposure leading to bioaccumulation and there are chances for pesticide residues to be transported to the lake from all paddy fields through the rivers. Sublethal concentrations of pesticides in the aquatic ecosystems for longer periods can cause cumulative accumulation in fishes and other organisms and as a consequence damage the organism itself and those who consume these organisms. Quinalphos was seen to be significantly more toxic as compared to monocrotophos, it is evident from the concentrations accumulated over 21 days. Organophosphate pesticides even at minimum concentrations in the aquatic environment tend to accumulate in fish tissues over long exposure periods, designating the onset of chronic toxicity.

REFERENCES


material, isomers, and formulations of endosulfan to the fish Chanwa punctate, Bull. Environ. Contam. Toxicol. 27 (1), 239-243