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STUDIES ON PESTICIDES RESIDUES IN FARM FISH IN PUNJAB (INDIA)

Nasir Hafiz Zargar | Project Manager, IWMP Reasi, Jammu and Kashmir. - Corresponding Author

Jatinder Paul Singh Gill | Director Research, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab.

ABSTRACT

The present study was undertaken to ascertain the levels of pesticide residues in farm fish (57) collected from twenty different organised fish farms in various districts of Punjab viz., Patiala, Bathinda, Ludhiana, Sangrur and Ropar. The pesticide usage is more in Bathinda as compare to other districts whereas the usage of these chemicals is less in Ludhiana and Ropar area. Residue levels of endosulphan sulphate, DDT, aldrin and cypermethrin in farm fish comprised 34, 21, 7 and 3 per cent respectively. p,p'-DDE was the most predominant metabolite of DDT detected in fish samples.

KEYWORDS | Pesticide, pesticide residues, fish and Punjab.

INTRODUCTION

Since time immemorial, man has been a victim of pest and diseases, which have destroyed his food, fiber and threatened his very existence. Hence a large variety of synthetic pesticides intended for destroying, repelling or inducing the harmful effects of pests is in use for agriculture and public health today (Hassan 2002). The indiscriminate and uncontrolled use of pesticides in agriculture leads to high level of pesticides in water bodies near to lands, rivers and estuarine bodies (Sarkar and Gupta 1988) resulting in high concentration in aquatic life especially fish, prawns, otter, eels and shrimps present in water bodies either by direct ingestion or by maintenance of solubility equilibrium with water. Pesticide residues also reach the aquatic environment through direct run off, leaching, careless disposal of empty containers, equipment washings etc. Pesticide enters a biological system mainly by three routes: aerial, terrestrial and aquatic. The rate of accumulation of pesticides is higher through aquatic route than through other routes because of chemical nature of pesticide, higher lipid solubility and lower water solubility. Pesticides can accumulate in the fish tissues either as a result of contaminated feed intake or through contact with pesticides in water, posing a hazard to public health through the consumption of fish.

MATERIALS AND METHODS

Chemicals

Analytical grade reagents viz. Acetone, hexane, acetonitrile were glass distilled before use. Sodium sulphate was heated for 2 hr in a muffle furnace at 400°C.Florisil (60-100 mesh) 200g treated with dichloromethane and acetone. After this it dried in hot air oven at 130°C for 2h. Florisil was used as such without any treatment. Analytical technical grade standards with 93- 99% purity for OCPs;organochlorine pesticides (HCH and its isomers, heptachlor, aldrin, fipronil, butachlor, dieldrin, DDT and its metabolites, endrin, β-endosulfan and endosulfan sulphate), Ops; Organophosphorus pesticides (chlorpyrifos, monocrotophos, dimethoate, phorate, fenitrothion, parathion-methyl, malathion, fenamiphos, profenphos, ethion, triazophos and phosalone) and SPs;synthetic pyretheroids (cypermethrin, permethrin, cyfluthrin, cyhalothrin, deltamethrin and fenvalerate) were used to detect the residues of these pesticides in the samples.

Sampling

The fish samples of different species were collected from 20 organized fish farms located in different districts of Punjab. All the samples were placed in plastic bags and transported in styrofoam boxes with recyclable ice and stored at -20ºC until processing. All the samples were processed in duplicate. Three samples of each species of fish weighing 400-500g were collected from different organized fish farms and two hundred and fifty gram meat of each fish species was collected from market and representative sample of 50g was taken for further processing.

Extraction and analysis

The extraction and clean up of fish samples were carried out by the method as described by Abdullah and Hassan (1990) with modification. The samples were thawed before extraction and only edible portion was taken for analysis. Samples thoroughly homogenize in a homogeniser. Thoroughly grounded sample take in a mortar and add 100g anhydrous sodium sulphate to combine with water present and to disintegrate sample. Extraction was done with petroleum ether, acetonitrile and dichloromethane. For organophosphrous pesticide residues, extraction was done with hexane by using the same procedure. The collected extracts were evaporated completely and reconstituted with n-hexane. This extract (2 µl) was injected into gas chromatograph (GC) equipped with electron capture detector (ECD) for OCPs and SPs detection and flame thermionic detector (FTD) for OPs detection. Analyte was identified by comparing the retention times and peak height/area with the reference standard which was run under similar operating conditions. The confirmation of pesticide residues was done by GC-Mass Spectrometer, in which a characteristic mass spectrum was obtained based on mass-charge ratio of a compound. The reagent and sample blank were extracted and analyzed to negate the false peaks in common. The trueness of the method used for extraction and estimation was validated by the processing of spiked fish, samples with standard pesticides at concentrations of 0.5 and 1 ppm. Average recoveries were between 80-92 %. The results are expressed in arithmetic mean value of pesticide residues with unit of ng g-1 on wet weight basis.

The formula used for the quantification of residues was:

$$\text{Residues (mg/kg)} = \frac{\text{Peak area of the sample x ng of insecticide standard injected x final volume of the sample extract(ml)}}{\text{Peak area of the standard x volume of the sample (µl) injected x weight of the sample(g)}}$$

RESULTS AND DISCUSSION

The fish samples were collected from twenty different organized fish farms of Punjab. The results of analysis of samples collected from different fish farms showed the presence of HCH isomers, DDT metabolites, aldrin, endosulfan, endosulfan sulphate, cypermethrin, chlorpyrifos and ethion residues. Mean Levels of residues (µg/g) in farm fish in various districts of Punjab were shown in Table 1.

Table 1: Mean Levels of residues (µg/g) in farm fish in different districts of Punjab.

Pesticides	Districts						
	Patiala (15)	Bathinda (12)	Sangrur (14)	Ludhiana (9)	Ropar (7)	Mean (57)	Range (57)
β-HCH	0.0188	0.0374	0.0154	0.004	0.0150	0.0181	0.004-0.0374
-HCH	0.0078	0.0068	0.0012	BDL	0.0006	0.0033	BDL-0.0078
-HCH	0.0266	0.044	0.0166	0.004	0.0167	0.0216	0.004-0.044
Aldrin	0.0010	0.0032	0.0120	BDL	0.0043	0.0041	BDL-0.0120
p,p'-DDE	0.0094	0.0452	0.0034	0.001	0.0013	0.0121	0.001-0.0452
p,p'-DDD	0.0024	0.0012	0.0006	BDL	BDL	0.0008	BDL-0.0024
-DDT	0.0118	0.0464	0.0040	0.001	0.0013	0.0129	0.001-0.0464
Endosulfan	BDL	BDL	0.0004	BDL	BDL	0.00008	BDL-0.0004
Endosulfan sulphate	0.0320	0.0646	0.0070	BDL	0.003	0.0213	BDL-0.0646
Cypermethrin	BDL	0.0034	0.0066	BDL	BDL	0.002	BDL-0.0066
Chlorpyrifos	BDL	0.0002	BDL	BDL	BDL	0.00004	BDL-0.0002
Ethion	0.0006	BDL	0.0004	BDL	BDL	0.0002	BDL-0.0006

The numerals in parentheses indicate number of samples; BDL –Below Detectable limit (0.0001)

The mean and range of -HCH residues in fish samples were 0.0216µg/g and 0.004-0.044µg/g, respectively. The concentrations of β- and -HCH residues were the most dominated among HCH isomers. The predominance of occurrence of β-HCH, the most persistent form is due to the isomerisation α-HCH and -HCH into β-HCH and also due to its high stability and is also resistant to enzymatic and metabolic degradation (Dhananjayan and Muralidharan 2010). HCH levels in present study were lower than the levels reported by Singh and Singh (2008) i.e. 2.181 µg/g in carp fishes of Uttar Pradesh. HCH contamination might have occurred through atmospheric transport from other parts of India, where large amount of technical HCHs are still being used and high concentrations were found in biota (Ramesh et al 1992). The presence of HCH isomers and DDT and its metabolites can be attributed to the use of these insecticides in agricultural as well as anti-malaria sanitary activities, carried out throughout the country (Pandit et al 2002). The lowest levels of -HCH residue were present in fish samples collected from Ludhiana district (0.004 µg/g) and highest levels of -HCH residue were present in fish samples collected from Patiala district (0.0266 µg/g).

DDT residues comprised of various metabolites like p,p'-DDE, DDD, o,p'-DDT and p,p'-DDT but only p,p'-DDE and p,p'-DDD residues were detected in the farm fish samples analyzed for pesticide residues. The predominance of occurrence of p,p'- DDE residue can be due to the conversion of p,p'-DDT to p,p'-DDE by mixed function oxygenase enzyme through metabolic functions. India banned DDT for agricultural purposes in 1989, but continues to use for malaria vector control and also used to control the Sand fly (Phlebotomus argentipes and P.papatasi) the vector of Kala-azar disease (Singh et al 1997). The dominance of either p,p'-DDE or both p,p'-DDT and p,p'-DDE in sediments was also recorded by Guruge and Tanabe (2001) from west coast of India. The lowest levels of -DDT residues were present in farm fish samples collected from Ludhiana district (0.001 µg/g) and highest levels of -DDT residues were present in farm fish samples collected from Bathinda district (0.0464 µg/g).

The mean and range of endosulfan and endosulfan sulphate residues in fish samples were 0.00008µg/g and BDL-0.0004 µg/g, 0.021µg/g and BDL-0.0646 µg/g, respectively. Begum et al (2009) also reported endosulfan i.e. 0.74 µg/kg in shrimps from various streams of Cauvery river, Karnataka. The restriction imposed on the use of aldrin, endosulfan is used widely in the management of termites and other insect pests in agriculture and can be the potential source of residues in animal feed and water (Ghosh et al 1999). Endosulfan is a xenoestrogen an endocrine disruptor, causing reproductive and developmental damage in animals and humans. It is a neurotoxic in insects and mammals. Endosulfan is highly toxic for aquatic organisms and has bioaccumulating effect especially in fish (Leena et al 2011). The lowest levels of endosulfan sulphate residues were present in fish samples collected from Ludhiana (BDL) and highest levels of endosulfan sulphate residues were present in fish samples collected from Bathinda district (0.0646 µg/g).

The mean and range of aldrin and cypermethrin residues in farm fish samples were 0.0041µg/g and BDL-0.012 µg/g, 0.002 µg/g and BDL-0.0066 µg/g, respectively. The lowest levels of aldrin residues were present in samples collected from Ludhiana (BDL) and highest levels of aldrin residues were present in samples collected from Sangrur district (0.012 µg/g). The lowest levels of cypermethrin residues were present in samples collected from Ropar (BDL) and highest levels of cypermethrin residues were present in samples collected from Sangrur district (0.0066 µg/g). Mean levels of residues (µg/g) in farm fish of Ropar and Sangrur.

Among the organophosphate pesticides, chlorpyrifos and ethion residues were found in the samples of fish. Five out 57 samples of farm fish analyzed showed the presence of ethion residues, while chlorpyrifos were found in 3 out of 57 samples. The mean and range of chlorpyrifos in fish samples were 0.00004µg/g and BDL-0.0002 µg/g, respectively. The highest levels of chlorpyrifos residues were present in samples collected from Bathinda district (0.0002 µg/g). The mean and range of ethion in fish samples were 0.0002 µg/g and BDL-0.0006 µg/g, respectively. The highest levels of ethion residues were present in samples collected from Patiala district (0.0006µg/g). Chlorpyrifos levels in present study were lower than the levels reported by Amaraneni (2002) i.e. 12.6µg/g in fish from fish farms in Kolleru Lake, India and also by Hernandez et al (1998) from Spanish Mediterranean coast in Gambusia affinis i.e. 339 ng/g.

CONCLUSION

The mean levels of residues in farm fish were HCH, endosulfan sulphate, DDT, chlorpyrifos, aldrin, cypermethrin, endosulfan and ethion: 0.022, 0.0213, 0.0129, 0.00004, 0.0041, 0.002, 0.00008 and 0.0002 µg/g, respectively. The present study showed that there is shift towards use of pesticides from organochlorine and organophosphate to synthetic pyrethroids.

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