Accumulation of Malathion in some Tissues of Heteropneustes Fossilis (Ham.) as Assessed by Thin Layer Chromotography

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Abstract -

This paper presents the qualitative detection of malathion in the liver, kidney, testes, ovary, brain and gills of the fresh water teleost, Heteropneustes fossilis by thin layer chromatography. Study was made after exposure of fish to a sublethal concentration of 60 ppm for 30 days.Malathion breaks down sequentially to three detectable metabolites. Malathion metabolites brings about internal metabolic distrurbances and histopathological changes in these organs. The results indicate an accumulation of malathion in these organs.

Key words - Thin layer Chromatography, Malathion Fish.

Introduction -

Although the chemistry and metabolism of malathion in various substances have been studied extensively (O' Brien 1967, Richard 1970, Post and Leasure 1974, Cook and Moore 1976), practically no residue data has been reported for malathion in fish tissues.Matathion, an organophosphorus insecticide a major lethal poison which is widely used because of its efficiency and also less persistence in the aquatic environment.The present research paper deals with the identification of malathion residues in different tissues of the fresh water teleost Heteropneustes fossilis (HAM.) by thin layer chromatography and the main aim of the study has been to get information on distribution of malathion residues in fish organs.(Dubale and Shah, 1979; Pandey and Shukla, 1982; Singh and Sahai, 1986a; Shrivastava and Shrivastava 1984; Fulton and Key, 2001 and Rico and Waichman 2011)

Materials and Methods - Live specimens of *H.fossilis were* obtained from the local Sagar lake. Fish weighing 14 to 16g and 12 to 14 cm in length were used.They were acclimatized in the laboratory for a week before the experiment. The method of application of the pesticide and the bioassay used are the same as described by Singh and Sahai. (1984b). For thin layer chromatography the method of Walker and Beroza (1963) was used. Ten fish were taken each time at 96 h, 15 days and 30 days after exposure to malathion at a concentration of 6 ppm. Rf values of the spot were then calculated by the formula:

$$Rf = \frac{\text{Distance of Spot Centre From Start Point}}{\text{Distance of Solvent Front From Start Point}}$$

Results and Discussion - The results of qualitative determination of malathion residues in different tissues studied using solvent systems is given in chromatogram 1, 2 and 3

Chromatogram - 1
Layer - Silica gel - G
Solvent - Hexane : Acetone - (80:20)
Solvent front - 14 C.M.
Spray reagent - Palladium Chloride
Time - 50 Minutes

Days of exposure	Colour of the spot	Rf					
		Gill	Liver	Kidney	Testes	Ovary	Brain
96 h	Yellow	ND	0.37	0.33	ND	ND	0.32
15 Days	Yellow	0.38	0.39	0.36	0.32	0.33	0.36
	Bright yellow	0.84	0.83	0.85	0.64	0.82	0.64
	Faint yellow	ND	0.92	ND	ND	ND	0.82
30 Days	Yellow	0.38	0.39	0.32	0.33	0.36	0.33
	Bright yellow	0.84	0.86	0.80	0.64	0.83	0.63
	Faint yellow	ND	0.95	ND	ND	ND	0.85

Chromatogram - 2
Layer - Silica gel - G

Solvent Chlroform : Methanol - (90:10)

Solvent front - 18 C.M.

Spray reagent - Palladium Chloride

Time - 30 Minutes

96 h	Yellow	ND	0.35	0.37	ND	ND	0.35
15 Days	Yellow	0.38	0.36	0.38	0.37	0:37	0.35
	Brownish yellow	0.60	0.65	0.65	ND	0.60	0.62
30 Days	Yellow	0.39	0.35	0.37	0.37	0.38	0.35
	Yellow	0.65	0.66	0.65	0.88	0.65	0.66
	Brownish yellow	0.89	ND	ND	ND	ND	0.88

Chromatogram - 3

Layer - Silica gel - G

Solvent Chlroform : Methanol - (90:10)

Solvent front - 15 C.M.

Spray reagent - Palladium Chloride

Time - 30 Minutes

96 h	Yellow	ND	ND	ND	ND	ND	0.26
15 Days	Yellow	0.55	0.28	0.28	0.28	0.28	0.268
	Faint yellow	ND	0.56	0.55	0.54	0.56	0.56
30 Days	Yellow	0.55	0.28	0.28	0.27	0.28	0.29
	Yellow	0.80	0.55	0.55	0.54	0.56	0.55
	Faint yellow	ND	0.79	ND	ND	ND	0.77

ND - Not detectable

Malathion gives yellow, bright yellow, faint yellow or brownish yellow sports on the TLC plates depending on the solvent system used. Significant difference in the values of the different sports were observed.

With all the solvent systems used after 96h only one spot is visible in the liver, kidney and brain. This spot is not detectable in the gills and gonads. On 15 days exposure normally two sports are visible with varying Rf's in all the tissues. However the third faint

yellow spot appeared only in the TLB of liver, kidney and brain with the Hexane : Acetone solvent system. On 30 days exposure three distinct spots are visible in all the tissue chromatograms except in the Chloroform : Methanol solvent system where the third spot is not detectable in the chromatograms of the gonads and gills. This suggests that the residue in fish tissues vary greatly from organ to organ. It also shows that malathion breaks down sequentially to three detectable metabolites. Tissue samples indicate high residue concentration in liver, brain and kidney in the descending order.

Bhagwat and Ramchandran (1975) reported malathion A and B esterases in the liver of mouse. Nomeir and Douterman (1978) also found degradation of malathion in the liver of mouse. Manzie (1980) has studied the presence of five metabolites of malathion, viz. malathion monocarboxylic acid (MCA), malathion dicarboxylic and (DCA), dimethyl phosphorothioate (DMTP), dimethyl phosphorodithioate (DMDTP) and dimethyl phosphoric (DMP). Cooke et al. (1976) have reported that whole body analysis of pin fish (Lagodon rhoboide) exposed to malathion showed the presence of malathion mono and dicarboxylic acids, other metabolites were not detected. Cooke et al. (1976) have however shown that malathion is also rapidly absorbed in fish tissues.

In the present study TLC analysis shows that in the different tissues of heteropneustes fossilisbreaks up into three metabolites after 30 days exposure but after 15 days exposure two metabolites were present. However, with Hexane : Acetone which gave the best separation three metabolites were present even at 15 days exposure.

Knowledge of the location of pesticides in various tissues is important for understanding the route detoxification and degradation. The result of the present study shows that malathion breaks up into metabolites in the various tissues of clarias and consequently brings about internal metabolic disturbances and histopathological defects in these organs.

References -

1. Bhagwat, V.M. and B.V. Ramchandran : Malathion A and B easterases of mouse liver. I. Separation and properties, Biochem. Pharmacol., 24, 1713-1717 (1975).
2. Cooke, G.H. and J.C. Moore : Determination of malathion, malaxon and mono

and dicarboxylic acids of malathion in fish oyster and shrimp tissue. J. Agric. Food Chem., 24(3), 631-634 (1976).

3. Dubale, M.S. and Punita Shah : Histopathological lesion induced by malathion in the liver of channa punctatus. Ind. J. exp. Biol., 17(7), 693-697 (1979).

4. Manzei, C.M. Metabolism of pesticides update III. United States department of the interior fish and wild life service special scientific report wild life No. 232, Washington, D:C., 269-372 (1980).

5. M.H. Fulton and P.B. Key : Acetyl cholinesterase inhibition in esturine fish and invertebrate as an indicator of organophosphorus insecticide exposure and effects environmental toxicology and chemistry, Wily online Lib. (2001).

6. Nomeir, A.A. and W.C. Douterman : In vitro degradation of malathion by mouse liver. Biochem. Pharmacol., 27, 2975-2976 (1978).

7. Pandey, A.K. and L. Shukla : Effect of an organophosphorous insecticide malathion on the testicular histophysiology in Sarotheradon mossambicus. Nat. Acad. Sci. Letters, 5, 141-142 (1982).

8. Rico, A.V. Waichman et. Al. : Effects of malathon and Amerzonian Fresh water fishes : comparision of tropical and temperate species sensitivity distribution. Econtixicology ; Springer, Vol. 20 Page 625-634 (2011)

9. Shrivastava, J and A.K. Shrivastava : Histopathology of the gills of Channa gachua exposed to sublethal concentration of malathion and chlorodane. Proc. Sem. Pest Aq. Fau,. 37-44 (1984).

10. Singh. S. and S. Sahai : Effect of malathion on the mortality and behaviour of two fresh water teleosts. J. Environ Biol., 5(1), 23-28 (1984b).

11. Singh, S. and S. Sahai : Accumulation of malathion in the liver, kidney and gills of Puntius ticto (Ham.) as assessed by thin layer chromatography (TLC) J. Envir. Biol. 7(2) 107-112 (1986).

12. Walker, K.C. and M. Beroza : Thin layer chromatography for insecticide analysis. J. Assoc. Official Agri. Chemists., 46, 250 (1963).