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In Vivo Metabolism of Fenitrothion (0,0-dimethyl-0-(4-nitro-m-tolyl) phosphorothioate) in Fresh Water Teleost (*Tilapia mossambica*)

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Fenitrothion is widely used against a variety of insect pests leading to the exposure of non-target organisms as well. Miyamoto et al.(1979) reported fenitrothion as highly toxic to rainbow trout (Salmo gairdneri), blue gill, carp (Cyprinus carpio) and daphnia (Daphnia pulex). Its metabolic pathways in fish were found to be more or less similar to other species in vivo (Kanazawa 1975 and Miyamoto et al. 1979). The activities of drug metabolizing enzymes in aquatic organisms are reported lower than in mammals (Matsumura 1976, Wong et al. 1981 and Suguira et al. 1981). Since, data on bioaccumulation and biotransformation of phosphorothioate insecticide is lacking in fresh water teleost it was decided to study fenitrothion metabolism in Tilapia mossambica in order to understand the metabolic processes involved in the biotransformation of fenitrothion.

MATERIALS AND METHODS

Fenitrothion - 97 (technical) obtained commercially (M/s Rallis India Ltd., Secunderabad) was purified by column chromatography as described by Metcalf (1967). Fresh water teleosts purchased locally were acclimatized to laboratory conditions for a week before the experiment. Prestarved teleosts were divided into seven batches of ten each weighing 5 - 9 gm and measuring 5-7 cm in length. Six batches were exposed to 200 mg/kg body weight of fenitrothion for 24 hours as described by Mustafa et al. (1982). The fishes were provided feed ad libitum. The seventh batch served as control. After 24 hours fishes were sacrificed, liver, kidney and brain were quickly dissected out, washed thoroughly with M/15 phosphate buffer (pH 7.2) to remove blood and homogenised separately (1 : 4) in M/15 phosphate buffer.

Fenitrothion and its metabolites were extracted twice in 20 ml of benzene and ethyl acetate (1 : 10) by decantation and pooled extracts were concentrated on rotavapor (Buchi EL - 130 Model). Number of metabolites were determined by analytical TLC using chloroform as mobile phase. To separate fenitrothion and its metabolites by HPLC 10 ul of solvent extract was injected in HPLC under the following conditions : Bondapak CN 30 cm ID Column; solvent system : benzene, hexane and ethyl acetate : : 25 : 23 : 2; flow rate : 1.5 ml/min; Pressure : 500 PSI; Detector : AVN 313 mm and sensitivity:IAVFS.

Solvent extract was loaded on preparative silica gel TLC for separation

of metabolites. After developing plates, bands were scrapped and eluted in benzene. The benzene was removed by rotavapor and the purity of each metabolite was confirmed by two dimensional TLC. Two dimensional TLC was also used to separate overlapping metabolites. All the mass spectra, unless stated otherwise, were recorded on a VG micromass 7070 double mass spectrometer and 70CV using the direct inlet system. The base peaks developed on spectrogram gave the molecular weight of the compound.

The laboratory raised culture of 1 - 4 week old flour beetles (Tribolium castaneum) on wheat flour and yeast powder (98 : 2) were used to test the toxicity of fenitrothion and its metabolites extracted from liver, brain and kidney. Several batches of ten beetles each were made. Three such batches were used to determine the toxicity of each metabolite or parent compound. Pre-determined aliquot of each metabolite in acetone was poured in the centre of glass petridish alongwith 1 ml of additional acetone in order to give uniform coating by rotating it to dryness. One batch of beetles was released on the surface of each petridish holding deposit of fenitrothion or its metabolites. The mortality data was recorded after 24 hours. LD_{50} values and standard errors were calculated by the method of probit analysis (Finney 1952).

RESULTS AND DISCUSSION

The observations on metabolism of fenitrothion - a phosphorothioate insecticide in liver, brain and kidney tissues of T. mossambica by using TLC technique is shown in Figure 1. Figures 2 - 4 indicated the analysis of metabolic extract on HPLC.

Figure 1 indicates 6, 5 and 3 metabolites in liver, kidney and brain extracts respectively.

Figure 2, 3 and 4 indicates 6, 5 and 3 peaks on chromatograms (HPLC) in liver, brain and kidney extracts respectively.

Table 1 shows the number of fenitrothion metabolites extracted from liver, kidney and brain, their R_f values and molecular weights. The metabolite II of molecular weight 261 corresponds to fenitrooxon. The other metabolites extracted from liver were identified as N-acetyl amino fenitrothion (IV) and fenitrothion (VI) of molecular weights as 288 and 277 respectively. The metabolites extracted from kidney were identified as desmethyl-N-acetyl amino fenitrothion (I) and desmethyl-N-acetyl amino fenitrooxon (II) of molecular weights 291 and 275 respectively. The metabolites from brain extract were identified as 3-methyl-4-nitro phenol (I) and desmethyl-N-acetyl amino fenitrooxon (III) of molecular weights 153 and 275 respectively.

Table 2 shows the relative toxicity of fenitrothion and its metabolites to adult flour beetles when exposed to deposits on glass surface for 24 hours indicated maximum toxicity of metabolite II of fish liver against flour beetles. Metabolite (II) was found to be several times more toxic than fenitrothion (Table 2). The other metabolites extracted from liver were less toxic than fenitrothion. The metabolite (II) of kidney was found to be more toxic than metabolite (III) followed by

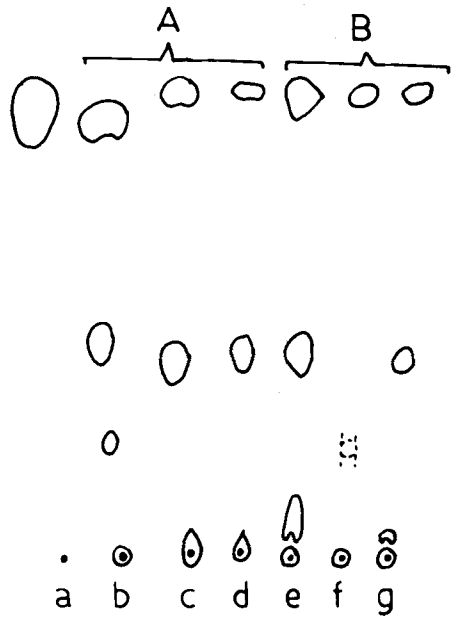

Figure 1 TLC spots of benzene (A) and ethyl acetate extract (B) using chloroform solvent system. a : fenitrothion, b & e : liver, c & f : brain, d & g : kidney

metabolites (I, V and IV). Similarly, the metabolite (II) of brain was more toxic to flour beetles than metabolite (I) and metabolite (III). The present data on in vivo metabolism of fenitrothion is indicative of oxidative-desulfuration, O-demethylation, cleavage of P-O-aryl linkage and amino derivatives of N-acetyl fenitrothion and fenitrooxon as routes of metabolic pathway in liver, brain and kidney of T. mossambica. Kanazawa (1975) and Miyamoto et al. (1979) reported the same pathway in rainbow trout and carp (Cyprinus carpio). Several amino derivatives of fenitrothion were also demonstrated by Takimoto et al. (1976, unpublished work) in carp (C. carpio Linneaus) tissues together with the nitro containing compounds.

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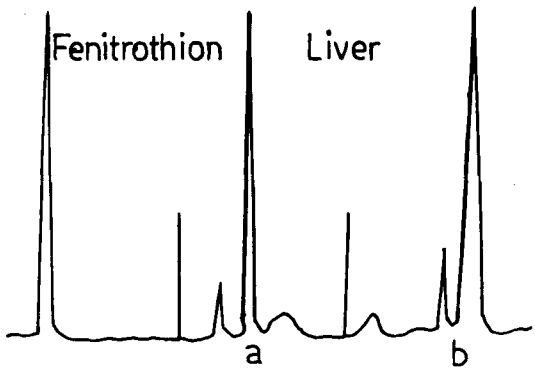

Figure 2 Chromatograms of benzene (a) and ethyl acetate extracts (b) of fenitrothion and fish liver samples using Bondapak CN and benzene, hexane, ethyl acetate (25:23:2)- as eluent.

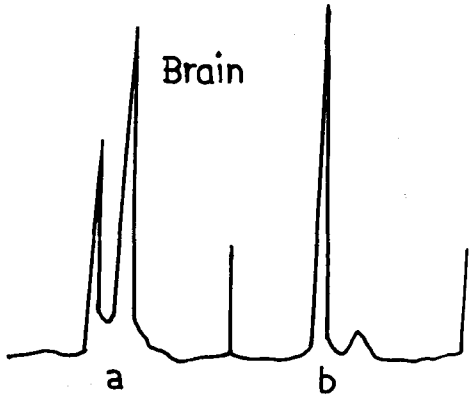

Figure 3 Chromatograms of benzene (a) and ethyl acetate extracts (b) of fish brain sample.

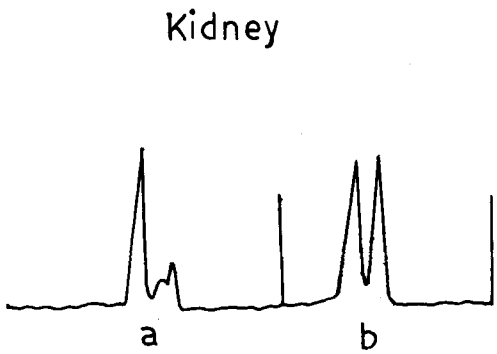

Figure 4 Chromatogrms of benzene (a) and ethyl acetate extracts (b) of fish kidney sample.

Table 1 Metabolites of fenitrothion formed in liver, brain and kidney of T. mossambica

Source	Metabolite	R_f value	Molecular weight	Name of metabolite
Liver	I	0.93	Ud.	-
	II	0.34	261	Fenitrooxon
	III	0.23	Ud.	-
	IV	0.07	288	N-acetyl amino fenitrothion
	V	0.94	Ud.	-
	VI	0.36	277	Fenitrothion
Brain	I	0.92	153	3-methyl-4-nitrophenol
	II	0.39	Ud.	-
	III	0.06	275	Desmethyl-N-acetyl amino fenitrooxon
Kidney	I	0.95	291	Desmethyl-N-acetyl amino fenitrothion
	II	0.43	275	Desmethyl-N-acetyl amino fenitrooxon
	III	0.04	Ud.	-
	IV	0.96	Ud.	-
	V	0.05	Ud.	-

Ud. represent Unidentified metabolites.

Table 2. Toxicity of fenitrothion and its metabolites to adult T. casteneum.

Insecticide/ Source	Metabolite	LD_{50} (mg/cm^2)	Relative Toxicity
Fenitrothion	-	0.05	-
Liver	I	5.00	200.00
	II	0.008	133333.33
	III	0.01	71428.57
	IV	25.00	40.00
	V	0.48	2083.33
	VI	1000.00	1.00
Brain	I	1000.00	1.00
	II	600.00	1.66
	III	1000.00	1.00
Kidney	I	1000.00	1.00
	II	99.00	1.01
	III	600.00	1.66
	IV	1000.00	1.00
	V	1000.00	1.00

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