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Comparative Study on Individual and Combined Effects of Dimecron and Ziram on Carbohydrate Metabolites in Liver, Muscle, Heart, and Blood of a Freshwater Teleost, *Sarotherodon mossambicus* (Peters)

P. Thangavel,[1] H. Nivedhitha,[2] M. Ramaswamy[2]

[1] Department of Environmental Sciences, Bharathiar University, Coimbatore-641 046, Tamil Nadu, India
[2] Department of Zoology, Government Arts College (Autonomous), Coimbatore-641 018, Tamil Nadu, India

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Dimecron or phosphamidon ($C_{10}H_{19}O_5$ NCIP) is a systemic insecticide and widely used for the control of *Scirpophaga incertulas* and other paddy pests. Ziram (zinc bis dimethyldithiocarbamate) is a wide range fungicide, effective against many fungal diseases such as rice sheath rot (*Sarocladium oryzae* Gams&Hawks), apple scab (*Venturia inaequalis* (Cooke) Wint.), apple pink disease (*Corticum salmonicolor* (Dast.) Berk.&Br.) and apple blight (*Erwinia amylovora* Winsl.). Inclusion of metal-based fungicides is known to increase pesticidal activity (Ramaswamy et al. 1996). Spraying of the organophosphate pesticide, dimecron in combination with the zinc-based carbamate fungicide, ziram (cuman L) provides a persistent effect of dimecron on different types of pest ("Cuman L" published by M/s. Hindustan CIBA-GEIGY Ltd., Mumbai). A survey of the literature indicates that the information on the effects of mixtures of pesticides on biochemical aspects of fish are very meagre (Oruc and Uren 2000). However, there exists no study on the metabolite levels of the fish exposed to mixtures which show combined toxicity against crop pests. Hence, an attempt has been presently made to investigate the individual and combined effects of dimecron and ziram on the levels of carbohydrate metabolites (glucose, glycogen and lactic acid) in liver, muscle, heart and blood of a freshwater edible fish, *Sarotherodon mossambicus* (Peters).

MATERIALS AND METHODS

A bulk sample of *Sarotherodon mossambicus* was procured from freshwater lakes in and around Coimbatore city and maintained in large tanks for one month. The fish were fed with boiled chicken eggs on alternate days. Water in the tank was renewed after each feeding and the fish were checked for infection regularly. A week before the commencement of the investigation, a randomly selected batch of healthy fish (size range: 8-12 g) was carefully transferred to a small cement cistern and maintained under laboratory conditions (29±1° C). During this period, the fish were fed daily and water was renewed daily. A day before the start of the experiment, feeding was discontinued.

For studying the individual effects of dimecron and ziram, fish were exposed for different periods to 0.002 mL L^{-1} of dimecron and 0.008 mL L^{-1} of ziram which

Correspondence to: P. Thangavel

formed the component composition of a sub-lethal 1:5 dimecron-ziram mixture used by Dhanalakshmi (1992). Fish were also exposed to sub-lethal concentration (0.01 mL L^{-1}) (Dhanalakshmi 1992) of 1:5 dimecron-ziram mixture to study the combined effects *in vitro*. Twenty fish were exposed to every individual concentration and individual period of exposure (for treatment and control) in rectangular glass tanks (120x60x50 cm) with 200 L water (a total of 18 tanks were maintained). The test solutions (containing individual dimecron, ziram and their mixture) were renewed every 12 hr (EPA 1975) in order to maintain constant dissolved oxygen concentration.

Six surviving fish from each of control, individual dimecron, ziram and dimecron-ziram exposure tank were killed (by a blow on the head). Levels of glycogen and lactic acid were estimated in muscle (lateral skeletal muscles), liver and heart tissues, while glucose and lactic acid levels were estimated in blood (collected by directly puncturing the branchial vessels in the opercular chamber) of control and pesticide/fungicide-treated fish at each period of exposure. Glucose content of the blood together with the glycogen levels of different tissues were estimated by the method of Kemp and Kits (1954). Lactic acid levels of blood and tissues were estimated following the method of Barker and Summerson (1941). The changes in glucose, glycogen and lactic acid levels (in different tissues) of pollutant-exposed fish versus the controls were calculated as percentages and their significance were analysed by Student's 't' test (Steel and Torrie 1960). Data were also analysed statistically by ANOVA or 'F' test using one-way classification (Steel and Torrie 1960) and Duncan's Multiple Range Test (DMRT) (Alder and Roessler 1977).

RESULTS AND DISCUSSION

The results obtained in the present study (Tables 1-4) reveal that the individual dimecron and ziram exposures as well as the dimecron-ziram combined exposure caused apparent changes in the levels of carbohydrate metabolites (glucose, glycogen and lactic acid) in different tissues of *S. mossambicus*.

A comparative observation of the percent change (from control levels) in the levels of carbohydrate metabolites in the liver of pollutant-exposed *S. mossambicus* for different periods (24, 48 and 72 hr) (Table 1) with those in muscle, heart and blood (Tables 2, 3 and 4) showed that the individual and combined effects of dimecron and ziram are more pronounced in the liver tissue. The severe lactic acidosis (accumulation of lactic acid) in the liver tissue, particularly during prolonged combined exposure as well as during 48 and 72 hr of individual and combined exposures, indicates that the pollutants caused severe hypoxia in the fish. Such a severe hypoxia (as shown by reduced oxygen uptake) was reported in *S. mossambicus* exposed to individual dimecron and cuman L (≡ziram) and a mixture of dimecron and cuman L (Ramaswamy et al. 1996). Decreases in glycogen levels together with increased accumulation of lactic acid in the liver of fish is indicative of the operation of anaerobic breakdown of stored glycogen to meet the energy demand under pollutant stress. The higher magnitude of lactic acid accumulation in liver under combined exposure compared to that of

Table 1. Levels of carbohydrate metabolites in the liver of *Sarotherodon mossambicus* under individual and combined exposure to dimecron and ziram for different periods. Values are means of 6 observations. Percent change from control levels are given in parentheses.

Carbohydrate metabolites		Dimecron			'F' value	Ziram			'F' value	Dimecron-Ziram			'F' value
		24 hr	48 hr	72 hr		24 hr	48 hr	72 hr		24 hr	48 hr	72 hr	
Glucose ($mg\ g^{-1}$)	C	2.21^{a}	2.38^{a}	2.37^{a}	1.24	2.20^{a}	2.20^{a}	2.22^{a}	1.41	2.23^{a}	2.25^{a}	2.28^{a}	1.13
	E	2.24^{c} (+1) NS	2.45^{b} (+3) NS	1.58^{d} (-33) S	3.89	4.14^{b} (+88) HS	1.84^{d} (-16) S	2.62^{c} (+18) S	4.15	1.75^{d} (-22) S	2.12^{c} (-6) NS	2.59^{b} (+14) S	3.58
Glycogen ($mg\ g^{-1}$)	C	3.58^{a}	3.60^{a}	3.58^{a}	1.37	3.60^{a}	3.57^{a}	3.60^{a}	1.02	3.54^{a}	3.57^{a}	3.59^{a}	1.21
	E	0.73^{b} (-80) HS	0.63^{d} (-83) HS	0.67^{c} (-81) HS	3.93	2.01^{b} (-44) S	0.61^{d} (-83) HS	0.65^{c} (-82) HS	4.08	0.54^{d} (-85) HS	0.57^{c} (-84) HS	0.70^{b} (-81) HS	3.73
Lactic acid ($mg\ g^{-1}$)	C	0.19^{a}	0.19^{a}	0.21^{a}	1.17	0.21^{a}	0.20^{a}	0.19^{a}	1.08	0.17^{a}	0.18^{a}	0.19^{a}	1.01
	E	0.39^{d} (+105) HS	0.49^{c} (+158) HS	0.81^{b} (+286) HS	4.16	0.35^{d} (+67) S	1.12^{b} (+460) HS	0.39^{c} (+105) HS	4.24	3.92^{b} (+2206) HS	1.13^{d} (+528) HS	1.70^{c} (+795) HS	6.31

C- Control; E- Exposed

F (0.05) = 3.63; F (0.01) = 6.22

Percentage difference from control, + = increase; - = decrease

Across each row, mean values followed by a common letter are not significantly different at the 5 % level by DMRT

S-Significant, $P<0.05$; HS-Highly significant, $P<0.01$; NS-Not significant, $P>0.05$ (based on 't' test)

Table 2. Levels of carbohydrate metabolites in the muscle of *S. mossambicus* under individual and combined exposure to dimecron and ziram for different periods. Values are means of 6 observations. Percent change from control levels are given in parentheses.

Carbohydrate metabolites		Dimecron			'F' value	Ziram			'F' value	Dimecron-Ziram			'F' value
		24 hr	48 hr	72 hr		24 hr	48 hr	72 hr		24 hr	48 hr	72 hr	
Glucose ($mg\ g^{-1}$)	C	1.71^{a}	1.78^{a}	1.78^{a}	1.08	1.79^{a}	1.75^{a}	1.78^{a}	1.32	1.82^{a}	1.80^{a}	1.77^{a}	1.24
	E	1.54^{c} (-10) S	1.54^{c} (-14) S	1.59^{b} (-11) S	3.78	1.59^{c} (-11) S	1.62^{b} (-7) NS	1.54^{d} (-13) S	3.82	1.56^{d} (-14) S	1.64^{c} (-9) NS	1.79^{b} (+1) NS	3.92
Glycogen ($mg\ g^{-1}$)	C	0.51^{a}	0.53^{a}	0.52^{a}	0.98	0.52^{a}	0.57^{a}	0.55^{a}	1.16	0.50^{a}	0.51^{a}	0.52^{a}	1.17
	E	0.49^{c} (-4) NS	0.53^{b} (0) NS	0.49^{c} (-6) NS	3.68	0.50^{b} (-4) NS	0.50^{b} (-12) S	0.48^{c} (-13) S	3.91	0.49^{d} (-2) NS	0.53^{b} (+4) NS	0.52^{c} (0) NS	4.02
Lactic acid ($mg\ g^{-1}$)	C	1.05^{a}	1.02^{a}	1.05^{a}	1.21	1.03^{a}	1.07^{a}	0.94^{a}	1.21	1.07^{a}	1.03^{a}	0.99^{a}	1.06
	E	1.39^{b} (+32) S	0.97^{d} (-5) NS	1.03^{c} (-2) NS	3.72	0.50^{d} (-51) S	0.66^{b} (-38) S	0.65^{c} (-31) S	4.06	3.31^{c} (+209) HS	0.68^{d} (-34) S	5.47^{b} (+453) HS	4.22

C- Control; E- Exposed

F (0.05) = 3.63; F (0.01) = 6.22

Percentage difference from control, + = increase; - = decrease

Across each row, mean values followed by a common letter are not significantly different at the 5 % level by DMRT

S-Significant, $P<0.05$; HS-Highly significant, $P<0.01$; NS-Not significant, $P>0.05$ (based on 't' test)

Table 3. Levels of carbohydrate metabolites in the heart of *S. mossambicus* under individual and combined exposure to dimecron and ziram for different periods. Values are means of 6 observations. Percent change from control levels are given in parentheses.

Carbohydrate metabolites		Dimecron			'F' value	Ziram			'F' value	Dimecron-Ziram			'F' value
		24 hr	48 hr	72 hr		24 hr	48 hr	72 hr		24 hr	48 hr	72 hr	
Glucose (mg g^{-1})	C	1.93^{a}	1.95^{a}	1.96^{a}	1.01	1.97^{a}	1.94^{a}	1.91^{a}	1.24	1.92^{a}	1.95^{a}	1.94^{a}	1.37
	E	1.53^{d} (-21) S	1.56^{c} (-20) S	1.97^{b} (+1) NS	4.52	1.36^{d} (-31) S	1.58^{c} (-19) S	1.61^{b} (-16) S	4.27	1.59^{d} (-17) S	1.67^{b} (-14) S	1.65^{c} (-15) S	3.86
Glycogen (mg g^{-1})	C	0.49^{a}	0.50^{a}	0.47^{a}	0.98	0.49^{a}	0.49^{a}	0.51^{a}	1.63	0.51^{a}	0.47^{a}	0.48^{a}	1.09
	E	0.46^{c} (-6) NS	0.46^{c} (-8) NS	0.50^{b} (+6) NS	4.17	0.46^{d} (-6) NS	0.47^{c} (-4) NS	0.48^{b} (-6) NS	4.36	0.48^{d} (-6) NS	0.51^{c} (+9) NS	0.54^{b} (+13) S	4.24
Lactic acid (mg g^{-1})	C	0.21^{a}	0.17^{a}	0.22^{a}	0.87	0.24^{a}	0.24^{a}	0.26^{a}	1.17	0.21^{a}	0.27^{a}	0.26^{a}	0.84
	E	0.23^{c} (+10) NS	0.51^{b} (+200) HS	0.22^{d} (0) NS	3.98	0.12^{d} (-50) S	0.60^{b} (+150) HS	0.38^{c} (+46) S	4.12	0.80^{b} (+281) HS	0.50^{c} (+85) HS	0.37^{d} (+42) S	6.36

C- Control; E- Exposed

F (0.05) = 3.63; F (0.01) = 6.22

Percentage difference from control, + = increase; - = decrease

Across each row, mean values followed by a common letter are not significantly different at the 5 % level by DMRT

S-Significant, P<0.05; HS-Highly significant, P<0.01; NS-Not significant, P>0.05 (based on 't' test)

Table 4. Levels of carbohydrate metabolites in the blood of *S. mossambicus* under individual and combined exposure to dimecron and ziram for different periods. Values are means of 6 observations. Percent change from control levels are given in parentheses.

Carbohydrate metabolites		Dimecron			'F' value	Ziram			'F' value	Dimecron-Ziram			'F' value
		24 hr	48 hr	72 hr		24 hr	48 hr	72 hr		24 hr	48 hr	72 hr	
Glucose (mg mL^{-1})	C	0.97^{a}	0.90^{a}	0.91^{a}	1.14	0.92^{a}	0.89^{a}	0.89^{a}	1.23	0.92^{a}	0.92^{a}	0.92^{a}	0.97
	E	0.81^{b} (-16) S	0.79^{d} (-12) S	0.80^{c} (-12) S	4.62	0.76^{d} (-17) S	0.78^{b} (-12) S	0.77^{c} (-13) S	4.32	0.78^{d} (-15) S	0.82^{b} (-11) S	0.80^{c} (-13) S	4.12
Lactic acid (mg 100 mL^{-1})	C	13.72^{a}	13.41^{a}	13.58^{a}	0.97	13.91^{a}	13.88^{a}	13.82^{a}	1.43	13.90^{a}	13.89^{a}	13.95^{a}	1.16
	E	9.74^{b} (-29) S	12.05^{b} (-10) S	16.66^{c} (+23) S	3.72	4.23^{d} (-70) HS	21.22^{b} (+53) S	13.46^{c} (-3) NS	3.68	69.74^{b} (+402) HS	14.36^{d} (+3) NS	18.07^{c} (+30) S	6.27

C- Control; E- Exposed

F (0.05) = 3.63; F (0.01) = 6.22

Percentage difference from control, + = increase; - = decrease

Across each row, mean values followed by a common letter are not significantly different at the 5 % level by DMRT

S-Significant, P<0.05; HS-Highly significant, P<0.01; NS-Not significant, P>0.05 (based on 't' test)

individual exposures is suggestive of the fact that the mixture of pesticides is more toxic to the fish.

From Table 2, it is discernible that muscle showed decreased glycogen level but without corresponding elevation in lactic acid content. The observed drop in muscle lactic acid level, despite glycogen utilization (particularly during 24 and 48 hr) could be considered as an adaptation by the fish to avoid the onset of muscle fatigue (due to lactic acidosis) for increased activity of the fish under pollutant stress. The drop in lactic acid level is also indicative of either the probable conversion of the same into glycogen (by 'Cori cycle') which could be transported to other active tissues or the complete breakdown of lactic acid to generate energy to meet the pesticide stress. The lack of adaptive decline in muscle lactic acid levels, particularly during the combined exposure for 72 hr, also attests to the higher toxic nature of the pesticide mixture.

The lesser magnitude of change (compared to control) in levels of carbohydrate metabolites in heart tissue, particularly during 24 hr of individual dimecron and individual ziram exposure, indicate that the manifestation of toxicity of these two pollutants are lower on the heart tissue during the initial period of exposure. However, the increased accumulation of lactic acid in heart tissue under combined exposure for 24 hr indicates the comparatively higher toxic nature of the pesticide mixture. Individual as well as combined exposures showed toxicity on carbohydrate metabolism in heart tissues during prolonged exposures for 48 and 72 hr (Table 3).

Table 4 shows that the changes in carbohydrate metabolite levels in blood of the fish under individual and combined exposures are different during different periods of exposures without any significant trend. This probably suggest that the manifestation of toxicity is not uniform in blood as the blood served more for the transport of metabolites rather than an active site of metabolism.

In conclusion, it could be stated that the mixture of dimecron-ziram exerts more toxic effect on the fish compared to that of individual exposures to dimecron and ziram (by way of showing severe changes in the different types of carbohydrate metabolites). Further, the higher manifestation of toxicity of dimecron-ziram mixture is more pronounced in the carbohydrate metabolism of liver tissue than in muscle, heart and blood.

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