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Toxic Effects of Dimethoate and Carbaryl Pesticides on Carbohydrate Metabolism of Freshwater Snail *Lymnaea acuminata*

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Dimethoate (organophosphate) and carbaryl (carbamate) pesticides are commonly used now a day in agricultural fields for pest control and in freshwater bodies for control of aquatic weeds and weed fishes. The heavy use of these pesticides is the main cause for the continuous increase of the pesticide concentration in the aquatic media and in the biota. These pesticides reach in the water bodies by agricultural run off or irrigation (Li 1975), which cause adverse effects on the aquatic fauna. Both pesticides are the inhibitor of acetylcholinesterase (AChE) (O'Brien 1960). Metabolism and related enzyme systems of organisms is the chief target of these pesticides. Hypoxic condition occurs in aquatic organisms due to the presence of these pesticides in the water bodies, which cause destructive effect on carbohydrate metabolism, as carbohydrates are the chief and immediate source of energy (Umminger 1977).

Freshwater snail *Lymnaea acuminata* is numerically and energetically important primary consumers in many freshwater bodies (Burris et al. 1990). This snail is an important link of food chain in aquatic ecosystem. Effect on carbohydrate metabolism in this snail could damage the energy balance of the food chain of aquatic ecosystem. So, the aim of this investigation is to measure the effects of different sub-lethal exposure against dimethoate and carbaryl pesticides on carbohydrate metabolism of freshwater snail *Lymnaea acuminata*.

MATERIALS AND METHODS

Adult freshwater snail *Lymnaea acuminata* of almost uniform size range (36.4±1.8 mm shell height and 20.2±1.2 mm shell width) were collected from non-contaminated water bodies of Gorakhpur district of Uttar Pradesh and kept in glass aquarium containing 30 L dechlorinated tap water at least for 96h for acclimatization under laboratory conditions. Water was changed every day. Dead snails were removed as soon as possible to avoid water fouling. The snails were fed daily on washed and dried *Nymphaea* leaves during the whole acclimatization period. Dimethoate (O, O- dimethyl S- (N-methylcarbamoylmethyl) phosphorodithioate) and carbaryl (1- naphthyl – N – methylcarbamate) were used as organophosphate and carbamate pesticide, respectively. The LC_{50} values of dimethoate are 19.65 mg/L and 10.81 mg/L for 24h and 96h, respectively, while

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LC_{50} values of carbaryl are 20.05 mg/L and 14.19 mg/L for 24h and 96h, respectively for the freshwater snail *Lymnaea acuminata* (Srivastava and Singh 2001). Sub-lethal doses (i.e. 3.0 mg/L, 6.0 mg/L, 9.0 mg/L and 12.0 mg/L) of both the pesticides were used for biochemical experiments.

Desired amount of pesticides were mixed in glass aquarium containing 5 L dechlorinated tap water. Twenty snails were placed in the each aquarium. In control groups the water remains pesticide free. The water temperature was kept at 23±1°C during the whole experiment. No food was given to the snails during the course of experiment. Snails were treated for 24h and 96h and after the completion of treatment they were dissected and hepatopancreas (digestive organ) and ovotestis (reproductive organ) tissues were removed for biochemical analysis.

Glycogen was measured according to anthrone method of Van der Vies (1954). Homogenate (10 mg/ml, w/v) was prepared in 5% TCA. Pyruvate level was measured according to Friedemann and Haugen (1943). Homogenate (50 mg/ml, w/v) was prepared in 10% TCA. Lactate was estimated according to Barker and Summerson (1941), modified by Huckabee (1961). Homogenate (50 mg/ml, w/v) was prepared in 10% cold TCA. LDH activity was measured according to the Anon (1984) method. Homogenate (50 mg/ml, w/v) was prepared in 0.1M phosphate buffer (pH 7.5).

Each assay was replicated six times, values are expressed as mean ±SE of six replicates and Student's 't' test was applied to locate significant ($P<0.05$) differences between treated and control groups

RESULTS AND DISCUSSION

Data of biochemical analysis in the hepatopancreas and ovotestis tissue of the snail after the exposure to different sub-lethal doses is given in table 1 and 2. Dimethoate and carbaryl pesticides significantly alter the levels of glycogen, pyruvate and lactate and LDH activity in the hepatopancreas and ovotestis tissue (Tables 1 & 2).

Data clearly indicates that levels of glycogen and pyruvate reduced after the exposure in both the body tissues, while lactate level was increased after the exposure in both the body tissues. Glycogen level was reduced to 43% and 41% in hepatopancreas, while 59% and 56% in ovotestis tissue after exposure to 24h and 96h, respectively against dimethoate. In case of carbaryl reduction in glycogen level was 46% and 48% after 24h and 55% and 54% after 96h of treatment in hepatopancreas and ovotestis tissues, respectively. Pyruvate level was reduced to 17% in hepatopancreas, while 36% in ovotestis tissue after exposure to 24h and 96h against dimethoate. In case of carbaryl reduction in pyruvate level was 35% and 31% after 24h and 32% and 30% after 96h of treatment in hepatopancreas and ovotestis tissues, respectively. Lactate level was increased to 190% and 192% in hepatopancreas, while 193% and 200% in

ovotestis tissue after exposure to 24h and 96h, respectively against dimethoate. In case of carbaryl increment in lactate level was 212% and 215% after 24h and 221% and 223% after 96h of treatment in hepatopancreas and ovotestis tissues, respectively. Data also clearly shows that lactic dehydrogenase (LDH) activity was increased after the exposure in hepatopancreas and ovotestis tissues. Activity of lactic dehydrogenase was increased to 168% and 191% in hepatopancreas, while 202% and 212% in ovotestis tissue after exposure to 24h and 96h, respectively against dimethoate. In case of carbaryl increment in lactic dehydrogenase level was 170% and 216% after 24h and 194% and 226% after 96h of treatment in hepatopancreas and ovotestis tissues, respectively.

Depletion of glycogen may be due to the direct utilization for energy generation, a demand caused by pesticide-induced hypoxia. The fall in glycogen content in both the body tissues indicates its rapid utilization by the respective tissue as a consequence of pesticide toxic stress. Similar decrease in tissue glycogen content of liver was reported in blue gills treated with 2,4-D (PGBB) (Cope et al. 1970). Carbohydrates are the primary and immediate source of energy for fish exposed to stress conditions (Umminger 1977). The decrease in tissue glycogen level under pesticide treatment indicates great utilization of this substance to meet higher energy demands to mitigate pesticide toxic stress, since glycogen depletion is more prevalent under hypoxic condition. Snail exposed to sub-lethal concentrations of dimethoate and carbaryl required more energy, which was immediately obtained from the increased metabolism of glycogen. Decrement in pyruvate level is due to higher energy demand during pesticidal exposure. In consonance with the increase in lactate content there is a decrease in the pyruvate level and this trend has been observed in both the tissues. The decrease in pyruvate level suggests the possibility of a shift towards anaerobic dependence due to a remarkable drop in aerobic metabolism. The decrease in pyruvate could be due to its conversion to lactate, or due to its mobilization to form amino acids, lipids, triglycerides and glycogen synthesis in addition to its role as a detoxification factor (Sathya Prasad 1983). The level of tissue lactic acid is known to act as an index of anaerobiasis, which might be beneficial to the animal to tolerate hypoxic conditions. The increase in lactate corroborates with the corresponding decrease in pyruvate content of the tissues of snail exposed to dimethoate and carbaryl. The increase in lactate also suggests a shift towards anaerobiasis as a consequence of hypoxia created under pesticide toxic impact leading to respiratory distress (Domsche et al. 1971; Siva Prasada Rao 1980). Increased lactic dehydrogenase (LDH) activity evidenced the conversion of pyruvate to lactate under anaerobic condition occurred by both the pesticides. So all the biochemical study evidenced that dimethoate and carbaryl pesticides caused destructive effect on carbohydrate metabolism in the body tissues of freshwater snail *Lymnaea acuminata*.

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Table 1. Glycogen (GN) (mg/g), pyruvate (PV) (μmole/g), lactate (LT) (mg/g) and lactic dehydrogenase (LDH) activity (μmole/mg protein/h) in hepatopancreas (HP) and ovotestis (OT) tissues of freshwater snail *Lymnaea acuminata* after dimethoate exposure.

	Tissue	Exposure period	Control	Dimethoate dose			
				3.0 mg/L	6.0 mg/L	9.0 mg/L	12.0 mg/L
GN	HP	24h	2.72±0.03 (100)	2.42±0.06* (89)	2.08±0.05* (76)	1.78±0.03* (65)	1.17±0.02* (43)
		96h	2.74±0.07 (100)	2.36±0.07* (86)	2.03±0.04* (74)	1.69±0.04* (62)	1.12±0.03* (41)
	OT	24h	2.63±0.04 (100)	2.44±0.08 (93)	2.14±0.07* (81)	1.84±0.06* (70)	1.54±0.02* (59)
		96h	2.56±0.03 (100)	2.38±0.06* (93)	2.09±0.04* (82)	1.79±0.05* (70)	1.43±0.04* (56)
PV	HP	24h	1.10±0.016 (100)	0.82±0.012* (74)	0.59±0.009* (54)	0.32±0.007* (29)	0.19±0.014* (17)
		96h	1.09±0.016 (100)	0.79±0.014* (72)	0.55±0.011* (50)	0.30±0.014* (28)	0.18±0.013* (17)
	OT	24h	1.34±0.017 (100)	1.04±0.012* (78)	0.72±0.009* (54)	0.59±0.016* (44)	0.48±0.014* (36)
		96h	1.26±0.016 (100)	1.00±0.011* (79)	0.69±0.012* (55)	0.55±0.009* (44)	0.46±0.015* (36)
LT	HP	24h	2.58±0.07 (100)	2.92±0.08* (113)	3.14±0.11* (122)	4.06±0.09* (157)	4.89±0.46* (190)
		96h	2.57±0.04 (100)	2.98±0.10* (116)	3.28±0.06* (128)	4.17±0.19* (162)	4.94±0.05* (192)
	OT	24h	2.28±0.12 (100)	2.88±0.19* (126)	3.05±0.04* (134)	3.86±0.17* (169)	4.41±0.06* (193)
		96h	2.32±0.03 (100)	2.96±0.14* (128)	3.14±0.03* (135)	3.96±0.19* (171)	4.64±0.02* (200)
LDH	HP	24h	0.068±0.002 (100)	0.073±0.002* (107)	0.081±0.004* (119)	0.094±0.007* (138)	0.114±0.010* (168)
		96h	0.070±0.003 (100)	0.081±0.004* (116)	0.097±0.006* (139)	0.117±0.009* (167)	0.134±0.011* (191)
	OT	24h	0.048±0.001 (100)	0.053±0.003* (110)	0.059±0.002* (123)	0.065±0.008* (135)	0.097±0.009* (202)
		96h	0.049±0.002 (100)	0.057±0.004* (116)	0.073±0.005* (149)	0.092±0.006* (188)	0.104±0.011* (212)

*, Significant ($P<0.05$), when Student's 't' test was applied between control and treated groups. Values are mean ±SE of six replicates. Values given in parenthesis are percent change with control taken as 100%.

Table 2. Glycogen (GN) (mg/g), pyruvate (PV) (μmole/g), lactate (LT) (mg/g) and lactic dehydrogenase (LDH) activity (μmole/mg protein/h) in hepatopancreas (HP) and ovotestis (OT) tissues of freshwater snail *Lymnaea acuminata* after carbaryl exposure.

	Tissue	Exposure period	Control	Carbaryl dose			
				3.0 mg/L	6.0 mg/L	9.0 mg/L	12.0 mg/L
GN	HP	24h	2.64±0.08 (100)	2.38±0.07 (90)	1.98±0.09* (75)	1.64±0.06* (62)	1.21±0.04* (46)
		96h	2.63±0.06 (100)	2.34±0.08* (89)	1.87±0.03* (71)	1.56±0.04* (59)	1.26±0.06* (48)
	OT	24h	2.56±0.09 (100)	2.49±0.06 (97)	2.11±0.07* (82)	1.89±0.08* (74)	1.42±0.05* (55)
		96h	2.52±0.08 (100)	2.36±0.04 (94)	2.06±0.06* (82)	1.74±0.05* (69)	1.36±0.04* (54)
PV	HP	24h	1.13±0.014 (100)	0.98±0.049* (87)	0.73±0.044* (65)	0.57±0.017* (50)	0.39±0.016* (35)
		96h	1.09±0.014 (100)	0.92±0.036* (84)	0.80±0.020* (73)	0.58±0.014* (53)	0.34±0.011* (31)
	OT	24h	1.29±0.019 (100)	1.04±0.048* (81)	0.77±0.025* (60)	0.58±0.012* (45)	0.42±0.016* (32)
		96h	1.28±0.016 (100)	1.01±0.039* (79)	0.79±0.024* (62)	0.55±0.018* (43)	0.39±0.012* (30)
LT	HP	24h	2.59±0.05 (100)	2.96±0.07* (114)	3.30±0.11* (127)	5.02±0.17* (194)	5.49±0.14* (212)
		96h	2.57±0.03 (100)	2.98±0.18* (116)	3.18±0.13* (124)	4.87±0.12* (189)	5.52±0.15* (215)
	OT	24h	2.38±0.06 (100)	2.84±0.16* (119)	3.46±0.14* (145)	4.28±0.21* (180)	5.26±0.15* (221)
		96h	2.32±0.11 (100)	2.92±0.19* (126)	3.27±0.16* (141)	4.34±0.17* (187)	5.17±0.13* (223)
LDH	HP	24h	0.070±0.002 (100)	0.079±0.004* (113)	0.087±0.004* (124)	0.106±0.022* (151)	0.119±0.026* (170)
		96h	0.063±0.002 (100)	0.081±0.005* (128)	0.09±0.008* (143)	0.110±0.009* (175)	0.136±0.024* (216)
	OT	24h	0.049±0.003 (100)	0.062±0.007* (126)	0.071±0.005* (143)	0.089±0.016* (182)	0.095±0.017* (194)
		96h	0.050±0.003 (100)	0.064±0.005* (128)	0.073±0.008* (146)	0.090±0.012* (180)	0.113±0.021* (226)

*, Significant ($P<0.05$), when Student's 't' test was applied between control and treated groups. Values are mean ±SE of six replicates. Values given in parenthesis are percent change with control taken as 100%.

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