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Toxicity of Malathion and Carbaryl Pesticides: Effects on Some Biochemical Profiles of the Freshwater Fish *Colisa fasciatus*

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The fat solubility of pesticides allow them to concentrate in host tissues. Because pesticides are used on a large area annually, aquatic ecosystems in agricultural areas are often inadvertently dosed through over spray and runoff (Gruber and Munn 1998). Organophosphorus and carbamate pesticides act as anticholinesterase agents by binding to the esteratic site of the cholinesterase (ChE) enzyme. Dutta et al. (1996) reported that the gill of *Heteropneustes fossilis* was affected by a sub-lethal dose of malathion. Uno et al. (2001) observed the residual properties of malathion in the organs of the bivalves *Anodonta woodiana* and *Corbicula leana*. Carbamate pesticide caused high mortality in the brown garden snail *Helix aspersa* at very low doses (Schuytema et al. 1994) and they are injurious to the liver of fish (Gill et al. 1990). Organophosphorus and carbamate pesticides caused disruptive effects on carbohydrate and protein metabolism of the freshwater snail *Lymnaea acuminata* (Tripathi and Singh 2002; 2003).

Colisa fasciatus is the common larvivorous fish of south-east Asia (Sumodan and Kumar 1998) extensively used for biological control of mosquito larvae in freshwater. This fish is also eaten by poor people, especially in villages. So, the aim of this study was to examine the toxicity of malathion and carbaryl to the freshwater fish *Colisa fasciatus* and evaluate the effect of sub-lethal doses of these pesticides on biochemical profiles of the fish.

MATERIALS AND METHODS

Adult freshwater fish *Colisa fasciatus* of uniform size range (length 6.3±0.86 cm; width 3.6±0.49 cm; weight 2.4±0.24 gm) were collected from different water bodies of Gorakhpur district of Uttar Pradesh, India and kept in glass aquaria containing 50L of dechlorinated tap water for 7 days to acclimatize them to laboratory conditions. Water quality was measured according to the method of APHA et al. (1998). The temperature of the experimental water was 23±0.7°C, pH was 7.3±0.2, dissolved oxygen was 7.2±0.3 mg/L, free carbon dioxide was 5.9±0.6 mg/L and alkalinity was 107±7.8 mg/L. Water was changed every day. Dead fish were removed as soon as possible to avoid water fouling. Fishes were fed daily on commercial fish food manufactured by Tokyu, Japan. Technical grade malathion [(diethyl mercaptosuccinate, S-ester) O, O-dimethyl

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phosphorodithioate] (purity 94%) and carbaryl (1-naphthyl-N-methylcarbamate) (purity 99%) were used as the organophosphate and carbamate pesticides.

Toxicity assay: Ten fishes were kept in glass aquaria containing 25L of dechlorinated tap water. Fishes were exposed to four different concentrations of two pesticides for 96h. Concentrations of malathion and carbaryl were 2.0, 2.5, 3.0 and 3.5 mg/L; 8.0, 8.5, 9.0 and 9.5 mg/L, respectively. Pesticide doses were given as the final concentration (w/v) of aquatic ingredient in the test aquaria. Control fishes were kept in dechlorinated tap water only. Each set of experiment was replicated six times. Mortality was recorded every 24h during the observation period of 96h. The LC values (LC_{10}, LC_{50} and LC_{90}), upper and lower confidence limit (LCL and UCL at 95% confidence limits), slope value, 't' ratio and heterogeneity were calculated by POLO programme (Russell et al. 1977). The product momentum correlation coefficient was determined between exposure time and different values of LC_{50} (Sokal and Rohlf 1973).

Biochemical assay: Five fishes were treated to sub-lethal doses (1.0, 2.0 and 3.0 mg/L for malathion and 4.0, 6.0 and 8.0 mg/L for carbaryl). One set exposed for 24h and another exposed for 96h, and after the completion of treatment they were dissected and liver and muscle tissues were removed for biochemical assay. Glycogen was measured according to the anthrone method of Van der Vies (1954). The homogenate (10 mg/ml, w/v) was prepared in 5% trichloro acetic acid (TCA). Optical density was measured at 650 nm against a blank and the value expressed as mg/g of tissue. Pyruvate level was measured according to Friedemann and Haugen (1943). The homogenate (50 mg/ml, w/v) was prepared in 10% TCA. Optical density was measured at 540 nm against a blank and expressed as μmole/mg of tissue. Lactate was estimated according to Barker and Summerson (1941), as modified by Huckabee (1961). The homogenate (50 mg/ml, w/v) was prepared in 10% cold TCA. Optical density was measured at 560 nm against a blank and expressed as mg/g of tissue. Total protein was measured according to Lowry et al. (1951). The homogenate (50 mg/mL, w/v) was prepared in 10% TCA. Optical density was measured at 600 nm against a blank and expressed as μg protein/mg of tissue.

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

RESULTS AND DISCUSSION

Data for the toxicity assay is given in Table 1. There was a negative correlation between the LC values of pesticides (malathion and carbaryl) and exposure periods (i.e. LC values decreases with increase in exposure period). The LC_{50} value of malathion was decreased from 3.15 mg/L (24h) to 2.12 mg/L (96h), while it was decreased from 9.04 mg/L (24h) to 8.00 mg/L (96h) for carbaryl (Table 1). Data for the biochemical assays are given in Tables 2 and 3. Data shows that glycogen, pyruvate and total protein content decreased while lactate content was increased in the liver and muscle tissues of the fish *Colisa fasciatus*

after exposure to all the sub-lethal doses of malathion and carbaryl (Tables 2 and 3).

Table 1. LC values (LC_{10}, LC_{50} and LC_{90}), lower and upper confidence limit (LCL and UCL), slope value, 't' ratio, 'g' value and heterogeneity for the freshwater fish *Colisa fasciatus* exposed to malathion and carbaryl.

Exposure period	LC values (mg/L)	Limits		Slope value	't' ratio	'g' value	Hetero-geneity
		LCL	UCL				
Malathion (organophosphate)							
24h	LC_{10} = 1.89	1.51	2.12				
	LC_{50} = 3.15	2.93	3.49	5.75±1.02	4.38	0.12	0.28
	LC_{90} = 5.26	4.42	7.44				
48h	LC_{10} = 1.75	1.39	1.97				
	LC_{50} = 2.85	2.67	3.07	6.04±0.99	4.84	0.10	0.36
	LC_{90} = 4.64	4.03	6.02				
72h	LC_{10} = 1.59	1.29	1.80				
	LC_{50} = 2.43	2.27	2.58	6.98±1.02	5.04	0.08	0.52
	LC_{90} = 3.72	3.39	4.34				
96h	LC_{10} = 1.48	1.19	1.68				
	LC_{50} = 2.12	1.94	2.25	8.29±1.21	5.86	0.06	0.68
	LC_{90} = 3.02	2.83	3.35				
Carbaryl (carbamate)							
24h	LC_{10} = 7.45	6.65	7,84				
	LC_{50} = 9.04	8.82	9.38	9.25±3.11	5.02	0.24	0.16
	LC_{90} =10.97	10.26	12.77				
48h	LC_{10} = 7.39	6.84	7.72				
	LC_{50} = 8.59	8.39	8.76	12.79±3.19	5.48	0.33	0.10
	LC_{90} = 9.97	9.61	10.66				
72h	LC_{10} = 7.29	6.76	7.59				
	LC_{50} = 8.30	8.09	8.46	16.54±3.44	6.12	0.56	0.09
	LC_{90} = 9.47	9.21	9.91				
96h	LC_{10} = 7.25	6.77	7.53				
	LC_{50} = 8.00	7.78	8.15	19.88±4.69	6.36	0.73	0.09
	LC_{90} = 8.83	8.67	9.09				

Concentrations given are the final concentration (w/v) in the aquaria. Batches of ten fishes were exposed to four different concentrations of each pesticide. Each set of experiment was replicated six times. LCL = Lower confidence limit and UCL = Upper confidence limit. Regression coefficient showed that there was a significant ($P<0.05$) negative correlation between exposure time and different LC values.

Malathion and carbaryl are esterase inhibitor neurotoxicants, with acute cholinergic effect preceded by inhibition of acetylcholinesterase (Barber et al. 1999). Being neurotoxicants, they interfere with many vital physiological

Table 2. Glycogen, pyruvate, lactate and total protein content in muscle and liver tissues of the freshwater fish *Colisa fasciatus* after malathion exposure.

	Tissue	Exposure period	Control	Dimethoate dose 1.0 mg/L	2.0 mg/L	3.0 mg/L
Glycogen (mg/g)	Muscle	24h	1.76±0.42 (100)	1.58±0.32 (90)	1.37±0.25* (78)	1.19±0.19* (68)
		96h	1.72±0.44 (100)	1.46±0.28* (85)	1.31±0.25* (76)	1.08±0.17* (63)
	Liver	24h	2.06±0.53 (100)	1.79±0.43* (87)	1.52±0.32* (74)	1.11±0.18* (54)
		96h	1.98±0.50 (100)	1.64±0.33* (83)	1.39±0.31* (70)	0.95±0.11* (48)
Pyruvate (μg/mg)	Muscle	24h	1.52±0.36 (100)	1.47±0.23 (97)	1.26±0.24* (83)	1.06±0.20* (70)
		96h	1.55±0.30 (100)	1.35±0.23* (87)	1.21±0.22* (78)	0.96±0.17* (62)
	Liver	24h	1.37±0.32 (100)	1.23±0.19 (90)	1.12±0.20* (82)	0.90±0.14* (66)
		96h	1.32±0.33 (100)	1.08±0.21* (82)	0.89±0.19* (68)	0.69±0.13* (52)
Lactate (mg/g)	Muscle	24h	2.38±0.53 (100)	2.52±0.42 (106)	2.83±0.63* (119)	3.14±0.72* (132)
		96h	2.32±0.64 (100)	2.57±0.48 (111)	2.85±0.61* (123)	3.22±0.67* (139)
	Liver	24h	2.27±0.61 (100)	2.47±0.39 (109)	2.88±0.56* (127)	3.27±0.79* (144)
		96h	2.21±0.59 (100)	2.56±0.45* (116)	2.85±0.58* (129)	3.34±0.82* (151)
Total protein (μg/mg)	Muscle	24h	146.04±6.16 (100)	140.19±6.12 (96)	122.67±5.23* (84)	106.61±4.86* (73)
		96h	143.82±5.94 (100)	130.88±6.04 (91)	112.18±5.45* (78)	100.67±4.48* (70)
	Liver	24h	117.46±4.99 (100)	108.06±5.67 (92)	95.14±5.14* (81)	81.05±4.33* (69)
		96h	114.36±5.07 (100)	102.92±5.44 (90)	82.34±5.11* (72)	68.62±3.89* (60)

*, 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 represent percent of control values.

Table 3. Glycogen, pyruvate, lactate and total protein content in muscle and liver tissues of the freshwater fish *Colisa fasciatus* after carbaryl exposure.

	Tissue	Exposure period	Control	Dimethoate dose 4.0 mg/L	6.0 mg/L	8.0 mg/L
Glycogen (mg/g)	Muscle	24h	1.76±0.42 (100)	1.55±0.31* (88)	1.27±0.31* (72)	1.06±0.26* (60)
		96h	1.72±0.44 (100)	1.44±0.29* (84)	1.15±0.27* (67)	0.95±0.22* (55)
	Liver	24h	2.06±0.53 (100)	1.76±0.42* (85)	1.44±0.36* (70)	1.07±0.27* (52)
		96h	1.98±0.50 (100)	1.58±0.31* (80)	1.23±0.31* (62)	0.69±0.20* (44)
Pyruvate (µg/mg)	Muscle	24h	1.52±0.36 (100)	1.46±0.23 (96)	1.23±0.24* (81)	1.12±0.20* (74)
		96h	1.55±0.30 (100)	1.38±0.23* (89)	1.18±0.23* (76)	1.05±0.18* (68)
	Liver	24h	1.37±0.32 (100)	1.25±0.19 (91)	1.07±0.21* (78)	0.85±0.16* (62)
		96h	1.32±0.33 (100)	1.14±0.21* (86)	0.87±0.20* (66)	0.74±0.15* (56)
Lactate (mg/g)	Muscle	24h	2.38±0.53 (100)	2.57±0.42 (108)	2.88±0.62* (121)	3.24±0.70* (136)
		96h	2.32±0.64 (100)	2.64±0.48* (114)	2.99±0.64* (129)	3.27±0.71* (141)
	Liver	24h	2.27±0.61 (100)	2.54±0.39* (112)	2.86±0.55* (126)	3.13±0.75* (138)
		96h	2.21±0.59 (100)	2.63±0.47* (119)	3.03±0.59* (137)	3.40±0.86* (154)
Total protein (µg/mg)	Muscle	24h	146.04±6.16 (100)	141.66±6.17 (97)	125.59±5.43* (86)	115.37±4.45* (79)
		96h	143.82±5.94 (100)	133.75±6.12 (93)	115.06±5.65* (80)	104.99±4.49* (73)
	Liver	24h	117.46±4.99 (100)	111.59±5.23 (95)	95.14±5.34* (81)	83.39±4.44* (71)
		96h	114.36±5.07 (100)	102.92±5.67 (90)	89.20±5.24* (78)	72.05±3.92* (63)

*, 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 represent percent of control values.

functions (Rao and Rao 1983), and consequently alter the levels of various body constituents (Arasta et al. 1996; Begum and Vijayaraghavan 1999) in fishes. Inhibition of AChE resulted in accumulation of acetylcholinesterase (AChE), which causes twitching of muscle leading to tetanus and eventual paralysis of the muscle. Paralysis of respiratory muscle may lead to death. Due to the agents being neurotoxicants the physiology of several systems may be affected, which results in disturbance of metabolic systems of the fishes. Statistical analysis of the data on toxicity brings out several important points. The steep slope value observed in the toxicity study demonstrates that a small increase in concentration of pesticide cause a large mortality in the fish. A 't' ratio greater than 1.96 indicates that the regression is significant. The χ^2 test for goodness (heterogeneity) less than 1.0 denotes that, in the replicate test of random samples, the concentration response lines would fall within 95% confidence limits and thus the model fits the data adequately. The 'g' value indicates that the value of the mean is within the limit at all probability levels (90, 95, 99) (Rand and Petrocelli 1988).

Carbohydrates are the primary and immediate source of energy. Depletion of glycogen may be due to direct utilisation of these compounds for energy generation, a demand caused by pesticide-induced hypoxia. Under hypoxic conditions, fishes derive their energy from anaerobic breakdown of glucose, which is available to the cells by the increased glycogenolysis (Vincent et al. 1995). The decrease in pyruvate level suggests the possibility of a shift towards anaerobic dependence due to a remarkable drop in the aerobic segment. The decrease in pyruvate could be due to its conversion to lactate, or due to its mobilisation to form amino acids, lipids, triglycerides and glycogen synthesis, in addition to its role as a detoxification factor (Tripathi and Singh 2002). Pyruvate oxidation could provide electrons and ATP for nitrogen reduction. The increase in lactate is consistent with the corresponding decrease in pyruvate content. The increase in lactate also suggests a shift towards anaerobiosis as a consequence of hypoxia created by pesticide toxicity leading to respiratory distress (Domsche et al. 1971). Due to pesticide exposure a tissue may receive less oxygen, leading to severe tissue hypoxia. Huckabee (1958) reported that an upward trend in lactate in the tissues might be taken to indicate that oxygen supply to the tissues is not adequate for normal metabolic function. Development of such internal hypoxic conditions may be ultimately responsible for the shift to less efficient anaerobic metabolism, evidenced by the change in lactate content observed during this study.

Proteins are the most important and abundant macromolecules in living beings, which play a vital role in architecture and physiology of the cell and in cellular metabolism (Mommsen and Walsh 1992), and constitute about 50% of their dry weight. As constituents of the cell membrane, proteins have a major role in the interactions between intra and extracellular media. As enzyme, proteins participate in the intricately balanced subcellular functions.

After the carbohydrate, the next alternative source of energy is protein to meet the increased energy demand. Further, under stress conditions, the protein consumed by fishes is not stored in the body tissue (Baskaran and Palanichamy 1990) and

hence the treated fish mete out their extra energy requirements from body proteins, which are mobilized to produce glucose, which is made available for fishes by the process of gluconeogenesis (Vasanthi et al. 1990). The depletion of the protein fraction in body tissues may have been due to their degradation and possible utilization of degraded products for metabolic purposes. Decreased protein content may be attributed to the destruction/necrosis of cells and consequent impairment in protein synthesis machinery (Bradbury et al. 1987).

So both pesticides, malathion and carbaryl, are poisonous / toxic to the freshwater fish *Colisa fasciatus*. The sub-lethal doses of both pesticides significantly altered glycogen, pyruvate, lactate and total protein levels in the fish. Fish with less nutritional value are not good for human consumption.

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REFERENCES

APHA, AWWA, WPCF (1998) Standard method for the examination of water and wastewater. 20th ed APHA Washington DC

Arasta T, Bais VB, Thakur P (1996) Effect of Nuvan on some biochemical parameters of Indian catfish *Mystus vittatus*. J Environ Res 80: 80-83

Baskaran P, Palanichamy S (1990) Impact of agricultural (ammonium chloride) fertilizer on physiology and biochemistry of fresh water teleost fish, *Oreochromis mossambicus*. J Ecobiol 2: 97-106

Barber D, Correll L, Ehrich M (1999) Comparative effectiveness of organophosphorus protoxicant activating systems in neuroblastoma cell and homogenates. J Toxicol Environ Health 57: 63-74

Barker SB, Summerson WH (1941) The colorimetric determination of lactic acid in biological materials. J Biol Chem 138: 535-542

Begum G, Vijayaraghvan S (1999) Effect of acute exposure of the organophosphate insecticide Rogor on some biochemical aspect of *Clarius batrachus* (Linn). Environ Res 80: 80-83

Bradbury SP, Makim JR, Coats JR (1987) Physiological responses of rainbow trout, *Salmo gairdneri* to acute fenvalerate intoxication. Pestic Biochem Physiol 27: 275-285

Domsche S, Domsche W, Classen M (1971) Zum mechanisms deri Leber Zeuschadigung durch Alkaylphosphate. Natururissenchaflan 58: 575

Dutta HM, Munsi JSD, Roy PK, Singh NN, Adhikari S, Killius J (1996) Ultrastructural changes in the respiratory lamellae of the catfish *Heteropneustes fossilis* after sublethal exposure to malathion. Environ Pollut 92: 329-341

Friedemann TE, Haugen GF (1943) Pyruvic acid I. Collection of blood for the determination of pyruvic acid and lactic acid. J Biol Chem 144: 67-77

Gill TS, Pandey J, Tewari H (1990) Hepatopathotoxicity of three pesticides in a freshwater fish, *Puntius conchonis*. J Environ Sci Health 25: 653-664

Gruber SL, Munn MD (1998) Organophosphate and carbamate insecticides in agricultural waters and cholinesterase (ChE) inhibition in common crop (*Cyprinus carpio*). Arch Environ Contam Toxicol 35: 391-396

Huckabee WE (1958) Relationship of pyruvate and lactate during anaerobic metabolism. II. Exercise and formation of O_2-debt. J Clin Invest 37: 255-263

Huckabee WE (1961) Blood analysis, determination of lactic acid. In: Oser BL (ed) Hawk's Physiological Chemistry, 14th edition, Tata McGraw-Hill, New Delhi, p 1103

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent. J Biol Chem 193: 265-275

Mommsen TP, Walsh PJ (1992) Biochemical and environmental perspectives on nitrogen metabolism in fishes. Experientia 48: 583-593

Rand GM, Petrocelli SR (1988) Fundamentals of aquatic toxicology. Hemisphere, New York, p 415

Rao KSP, Rao RKV (1983) Regulation of phosphorylases and aldolases in tissues of the teleost (*Tilapia mossambica*) under methyl parathion impact. Bull Environ Contam Toxicol 31: 427-478

Russell RM, Robertson JL, Savin NE (1977) POLO: A new computer programme for probit analysis. Bull Entomol Soc America 23: 209-213

Schuytema GS, Nebeker AV, Griffis WL (1994) Effects of dietary exposure to forest pesticides on the brown garden snail *Helix aspersa* Müller. Arch Environ Contam Toxicol 26: 23-28

Sokal RR, Rohlf FJ (1973) Introduction to biostatistics. W.H. Freeman & Co. San Francisco, p 365

Sumodan PK, Kumar A (1998) Distribution and feeding efficacy of larvivorous fishes of Goa. Indian J Malar 35:163-170

Tripathi PK, Singh A (2002) Toxic effects of dimethoate and carbaryl pesticides on carbohydrate metabolism of freshwater snail *Lymnaea acuminata*. Bull Environ Contam Toxicol 68: 606-611

Tripathi PK, Singh A (2003) Toxic effects of dimethoate and carbaryl pesticides on protein metabolism of the freshwater snail *Lymnaea acuminata*. Bull Environ Contam Toxicol 70: 146-152

Uno S, Shiraishi H, Hatakeyama S, Otsuki A, Koyama J (2001) Accumulation characteristics of pesticide residue in organs of bivalves (*Anodonta woodiana* and *Corbicula leana*) under natural conditions. Arch Environ Contam Toxicol 40: 35-47

Van der Vies J (1954) Two methods for the determination of glycogen in liver. Biochem J 57: 410-416

Vasanthi R, Baskaran P, Palanichamy S (1990) Influence of carbofuran on growth and protein conversion efficiency in some fresh water fishes. J Ecobiol 2: 85-88

Vincent S, Ambrose T, Cyrill L, Selvanaygam M (1995) Biochemical responses of the Indian major carp, *Catla catla* (Ham.) to chromium toxicity. Indian J Environ Health 37: 192-196