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Phosphamidon induced alterations in the nitrogen metabolic profiles of penaeid prawn, *Penaeus indicus* during acute and chronic exposure

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Abstract. Changes in the nitrogen metabolic profiles of midgut gland, muscle and gill tissues were studied, following the exposure of *Penaeus indicus* to acute (2 day) and chronic (10, 20 and 30 day) exposure to sublethal concentration (0·2 ppm) of phosphamidon. The proteolytic activity of phosphamidon exposed prawn tissues was significantly elevated. All the phosphamidon exposed prawn tissues showed efficient mechanisms of ammonia detoxification in the chronically exposed prawns when compared to acutely exposed prawns. The survivability of these prawns in the polluted habitats might be due to the operation of compensatory adaptive mechanisms in the metabolic profiles such as increased biosynthesis of different kinds of proteins and also the detoxification and transformation of ammonia, the toxic nitrogenous end product of aquatic organisms.

Keywords. Phosphamidon; protease; glutamate dehydrogenase; *Penaeus indicus*.

1. Introduction

Aquatic pollution has been increasing at an alarming rate due to indiscriminate use of various types of pesticides (Matsumura *et al* 1972; Holden 1973). Today most of the alterations in the natural environment are contributed by the widespread usage of different formulations of organophosphorous (OP) insecticides when compared to the persistent and highly toxic organochlorine insecticides to several nontarget organisms (Butler 1966; Bookhout and Monroe 1977). Eventhough the OP insecticides are considered to be nonpersistent, they inhibit acetylcholinesterase (AChE) activity with a subsequent disruption in the nerve impulse transmission (O'Brien 1967) and which will further lead to some physiological and metabolic changes of several nontarget organisms, which include a variety of crustaceans (Bhagyalakshmi 1980; Avelin Mary 1984; Srinivasulu Reddy *et al* 1985a, b). Phosphamidon, an OP insecticide has a wide range of applications in agriculture as an effective systemic pesticide. The present investigation deals with the toxic impact of sublethal concentration of phosphamidon on the tissue nitrogen metabolism of a penaeid prawn *Penaeus indicus* during acute (2 day) and chronic (10, 20 and 30 day) exposure periods.

2. Material and methods

Penaeid prawns *P. indicus* (H Milne Edwards) were collected from the Buckingham canal near Thummalapenta seacoast, Kavali. Only intermolt uninjured prawns of the size 75 ± 5 mm and weight 2·5 ± 0·5 g were selected and acclimatized to laboratory for 1 week at constant salinity of 15 ± 1‰, pH 7·1 ± 0·2 and temperature 23 ± 2°C.

They were fed *ad lib*, diet of oilcake powder. The media in which they were placed was changed for every 48 h.

Technical grade phosphamidon (92% w/v) (0, 0-dimethyl-0-(1-methyl-2-chloro-2-diethyl-carbomoyl-vinyl) phosphate) obtained from CIBA-GEIGY, Bombay was used as test chemical. A stock solution of 1000 ppm phosphamidon (1 mg in 1 ml) was prepared and appropriate working concentrations were prepared by dilution with seawater. The concentrations selected ranged from 0·2–1·5 ppm with a difference of 0·2 ppm. LC_{50} value was found to be 0·9 ppm for 48 h. Laboratory acclimatized prawns were exposed to a sublethal concentration of 0·2 ppm as described earlier (Srinivasulu Reddy *et al* 1985a). In the present study, the sublethal concentration was selected as it closely approximates the levels found in the environment (Nimmo 1979). The prawns were divided into 5 batches viz control and acutely exposed (2 day exposed) and chronically exposed (10, 20 and 30 day exposed) groups. Equal number of prawns were kept untreated for the same period under similar conditions served as controls. Prawns (50) were sacrificed from each glass aquarium i.e., after 2, 10, 20 and 30 days of phosphamidon exposure. The midgut gland, muscle and gill tissues were isolated, rinsed in crustacean ringer. The protein content was estimated by Lowry *et al* (1951) using bovine serum albumin as standard. Neutral protease activity levels and free amino acid content were estimated by the method of Moore and Stein (1954). Glutamate dehydrogenase (GDH) was assayed by the method of Lee and Lardy (1965). Ammonia, urea and glutamine were estimated by the methods of Bergmeyer (1965), Natelson (1971) and Colowick and Kaplan (1957), respectively. The data were subjected to statistical analysis as per Bailey (1965).

3. Results and discussion

The data presented in tables 1–3 show the changes in certain parameters of midgut gland, muscle and gill tissue nitrogen metabolic profiles during acute and chronic exposure to a sublethal concentration of phosphamidon.

On acute exposure to subacute concentration of phosphamidon, the total protein content of the midgut gland, muscle and gill tissues was considerably reduced. The sucrose soluble protein content was not significantly, whereas the sucrose insoluble protein content was significantly reduced. The neutral protease activity levels was elevated with a concomitant increment in the free amino acid content. GDH activity levels was also reduced but changes in the levels of ammonia, urea and glutamine were insignificant.

On chronic exposure to a sublethal concentration of phosphamidon, all the tissues showed a significant increment in the total, sucrose soluble and insoluble protein contents. Neutral protease levels were found to be significantly elevated but considerable reduction was observed in the free amino acid content. GDH activity levels was also elevated, whereas ammonia and urea were significantly decreased but glutamine content was significantly increased.

Acute exposure of phosphamidon causes depletion in the total protein content and this decrease might be due to increased proteolytic activity or enhanced entry of various proteins into hemolymph (Sreenivasula Reddy *et al* 1983b). The increased neutral proteolytic activity levels suggestive of the onset of degradable activities in all the tissues of phosphamidon exposed (PE) prawns, due to acute toxic impact of

Table 1. Changes in certain parameters in selected tissues of control and Phosphamidon exposed prawn.

		Exposure time (days)			
		Acute	Chronic		
Tissue	Control	2	10	20	30
Total proteins					
Midgut gland	185·41 ± 14·49	121·18 ± 10·12	164·23 ± 18·44	288·13 ± 21·18	312·62 ± 16·18
Muscle	228·90 ± 20·34	165·24 ± 18·42	301·38 ± 29·44	334·04 ± 31·31	364·32 ± 26·22
Gill	112·15 ± 12·10	84·42 ± 6·75	140·83 ± 10·42	162·18 ± 11·75	170·23 ± 11·45
Soluble proteins					
Midgut gland	120·45 ± 10·31	101·34[a] ± 10·15	189·21 ± 14·18	202·17 ± 15·34	234·44 ± 18·36
Muscle	69·95 ± 7·58	61·14[a] ± 5·84	112·25 ± 12·39	123·18 ± 13·45	141·22 ± 14·84
Gill	73·18 ± 6·05	62·48[a] ± 5·85	94·82 ± 6·71	115·72 ± 7·18	130·42 ± 7·48
Insoluble proteins					
Midgut gland	64·15 ± 6·13	43·41 ± 5·03	91·34 ± 7·89	100·43 ± 10·15	121·52 ± 10·86
Muscle	148·75 ± 13·42	102·34 ± 12·89	202·11 ± 18·44	228·19 ± 15·49	252·15 ± 15·18
Gill	51·11 ± 4·12	30·05 ± 3·12	61·23 ± 5·02	73·43 ± 6·09	80·12 ± 6·81

Each value is mean of 6 individual observations ± indicates SD. Values in parentheses are per cent alterations over control. All values are statistically significant at $P < 0·001$ except [a]$P < 0·01$.
Values are expressed as mg/g wet wt.

Table 2. Changes in certain parameters in selected tissues of control and phosphamidon exposed prawn.

		Exposure time (days)			
		Acute	Chronic		
Tissue	Control	2	10	20	30
Protease (μ mol of tyrosine formed/mg protein/h)					
Midgut gland	1·19 ± 0·18	1·62 ± 0·20	1·94 ± 0·28	2·09 ± 0·31	2·26 ± 0·29
Muscle	0·85 ± 0·12	1·12 ± 0·25	1·29 ± 0·32	1·34 ± 0·28	1·46 ± 0·29
Gill	0·62 ± 0·11	0·72[a] ± 0·12	0·81 ± 0·13	0·93 ± 0·14	1·05 ± 0·15
Free amino acids (μ mol/g wet wt)					
Midgut gland	94·17 ± 8·42	141·28 ± 12·49	51·44 ± 4·53	38·19 ± 5·13	32·15 ± 4·12
Muscle	185·23 ± 15·45	244·23 ± 23·49	102·14 ± 18·42	98·09 ± 5·44	87·82 ± 6·03
Gill	60·18 ± 4·42	85·75 ± 5·81	51·22 ± 3·88	46·82 ± 3·81	34·12 ± 2·89
GDH (μ mol of formazan formed/mg protein/h)					
Midgut gland	1·43 ± 0·15	1·01 ± 0·12	1·83 ± 0·24	2·03 ± 0·21	2·38 ± 0·23
Muscle	0·78 ± 0·05	0·61 ± 0·08	0·96 ± 0·09	1·05 ± 0·13	1·31 ± 0·14
Gill	1·02 ± 0·12	0·86[b] ± 0·09	1·33 ± 0·12	1·43 ± 0·15	1·51 ± 0·17

Each value is mean of 6 individual observations ± indicates SD. Values in parentheses are per cent alterations over control. All values are statistically significant at $P < 0·001$ except [a]Not significant; $P < 0·01$.

phosphamidon, with a concomitant reduction in the total protein content. The sucrose insoluble protein content showed a significant reduction in its content suggestive of higher degree of proteolysis at the structural organization of PE prawn tissues viz midgut gland, muscle and gill. The total free amino acid content was increased significantly which might be due to increased proteolysis as indicated by the results tabulated in table 2 or due to decreased utilization in oxidative metabolic

Table 3. Changes in certain parameters in selected tissues of control and phosphamidon exposed prawn.

		Exposure time (days)			
		Acute	Chronic		
Tissues	Control	2	10	20	30
Ammonia					
Midgut gland	10·45 ± 1·21	11·52[a] ± 1·10	4·18 ± 0·55	3·94 ± 0·41	3·22 ± 0·40
Muscle	5·32 ± 0·84	6·02[a] ± 0·58	2·84 ± 0·61	2·71 ± 0·50	2·52 ± 0·48
Gill	6·08 ± 0·74	7·05[b] ± 0·71	4·05 ± 0·32	3·08 ± 0·25	2·91 ± 0·28
Urea					
Midgut gland	1·19 ± 0·23	1·08[a] ± 0·20	0·61 ± 0·13	0·54 ± 0·11	0·43 ± 0·12
Muscle	1·059 ± 0·152	0·992[a] ± 0·101	0·589 ± 0·085	0·554 ± 0·081	0·548 ± 0·088
Gill	0·814 ± 0·051	0·790[a] ± 0·060	0·515 ± 0·055	0·445 ± 0·051	0·401 ± 0·048
Glutamine					
Midgut gland	21·44 ± 20·35	24·15 ± 2·13	58·78 ± 8·15	63·41 ± 7·85	75·08 ± 6·73
Muscle	6·72 ± 0·89	7·02[a] ± 0·75	16·49 ± 1·42	19·34 ± 1·84	23·18 ± 2·04
Gill	10·08 ± 0·95	11·42[b] ± 1·13	17·84 ± 1·80	21·80 ± 1·95	29·13 ± 2·05

Each value is mean of 6 individual observations ± indicates SD. Values in parentheses are per cent alterations over control. All values are statistically significant at $P < 0.001$ except [a]Not significant; [b]$P < 0.02$; [c]$P < 0.05$. Values are expressed as μ mol/g wet wt.

reactions (Srinivasulu Reddy *et al* 1983a; Bhagyalakshmi *et al* 1984). A significant reduction in the activity levels of GDH, which forms an index of amino acid oxidation into Kreb's cycle, the decreased mobilization of amino acid into oxidation can be expected. Similar kind of observations were reported in crabs under sumithion toxicity (Sreenivasula Reddy *et al* 1982). The contents of ammonia, urea and glutamine were not significantly affected, indicative of the probability of least disturbance in the formation and excretion of nitrogenous end products of all the PE prawn tissues in response to acute exposure of phosphamidon.

A significant elevation in the total, sucrose soluble and insoluble protein content suggesting the onset of their increased protein biosynthesis of PE prawn tissues or decreased proteolytic activity. But the protease activity levels was found to be shown a significant increment. The total free amino acid content showed a considerable reduction, which might be due to increased operation of transamination reactions under pesticide stress (Bhagyalakshmi *et al* 1984). GDH activity levels was significantly increased. Inspite of increased oxidative deamination as evidenced by increased activity levels of GDH, ammonia content was shown to be considerably decreased. Most probably the mobilization of ammonia towards the formation of less toxic substances like urea or glutamine may be one of the adaptive mechanisms by which it detoxifies the ammonia present at the cellular environment of PE prawn tissues (Bhagyalakshmi 1980). But interestingly, urea showed a decrement indicative of urea biosynthesis from ammonia was ruled out.

From the present study it can be concluded that even the low concentration of phosphamidon was able to alter the tissue metabolic profiles of PE prawn tissues during acute (2 day) and chronic (10, 20 and 30 days) exposure. All the PE tissues have shown efficient mechanisms of detoxification by shifting its nitrogen metabolic patterns and also adopting compensatory mechanisms like increased biosynthesis of various kinds of proteins and also detoxification of toxic substances like ammonia to

relatively nontoxic substances will pave way for the survivability of these prawns in the phosphamidon contaminated environments.

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