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Manifestations Studies on Enzyme Profile of *Vibrio parahaemolyticus* MTCC451 Inoculated Black Tiger Prawn *Penaeus monodon*

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ABSTRACT

Vibriosis is one of the major pathogenic bacterial diseases in shrimp aquaculture. Since, the disease patterns differ and the causes of the diseases are multifactorial, the results of such diagnostic profile tests could be correlated to the differing patterns of the disease. The present study aimed to evaluate the metabolic profile (biochemical and enzymatic) of *V. parahaemolyticus* MTCC 451 inoculated black tiger prawn *P. monodon* (Fabricius). The injected *V. parahaemolyticus* MTCC451 resulted in the outbreak of vibriosis in the tested *P. monodon*. The levels of Alkaline phosphatase activity, Acid phosphatase activity, Lactate dehydrogenase activity, Glutamate Pyruvate Transaminase (GPT) activity, Glutamate Oxaloacetate Transaminase activity, Chitinase activity, Water content, Sodium, Potassium, Calcium, Ascorbic acid and Histamine in Haemolymph, Hepatopancreas and Body muscle of *P. monodon* control Vs tested were noted. The laboratory results of the present investigation, using the inoculum *Vibrio parahaemolyticus* MTCC451 revealed that these endemic bacterial populations might become opportunistic pathogens, when hydroecological conditions are altered in the farm ponds and could bring the fatal episodes of mass mortality.

Key words: Shrimp culture, vibriosis, *Vibrio parahaemolyticus*, histopathology, hydroecological conditions

INTRODUCTION

Shrimp culture represents an important and economically profitable venture and their production has grown enormously in recent years by intensive and semi-intensive methods of culture. Penaeid shrimps are one of the most important preferred species for culture in artificial impoundments (Sekar *et al.*, 2014). Approximately more than 5 million metric t of shrimps are annually produced but the current global demand for both the wild (naive) and farmed shrimps are approximately more than 6.5 million metric t per annum (Karthik *et al.*, 2014). To overcome this, many shrimp farms are being created throughout the world to solve this increasing food demands (FAO., 2012). However, fast development of these shrimp industries and intensive culture of these farms has created various ecological, economical and social issues. In normal, diseases in aquaculture practices are mostly caused by luminous bacteria *Vibrio* sp. and it has been referred as the largest economic loss in the shrimp aquaculture due to mass mortalities (Sivakumar *et al.*,

2012). Important aspects of Vibriosis in penaeid shrimp summarized by Lightner (1993) are as follows: (1) Infections may be chronic, sub acute and mortality may reach 100% in some cultured population, (2) Most out breaks may be the consequences of extreme stresses and opportunistic pathogens, (3) Larval, post larval and adult shrimp may be infected, (4) *Vibrio* species, which infect shrimp, are ubiquitous and have been reported from all major shrimp culture regions and (5) *Vibrio* species and strains differ markedly in their virulence for penaeids as they do for other hosts. The above aspects of Vibriosis disease and its manifestations warrant research studies to diagnose its symptoms, besides management and quality control criteria in shrimp production.

It is known that in animals, manifestation of the disease by pathogens involves the breakdown of homeostasis, when the latter organisms and their load reach beyond the threshold limit of tolerance of the host animals. The blood and tissue level metabolites and compounds maintain normal base levels in animals of well maintained and/or well acclimatised without stress conditions. Besides the diurnal and seasonal variations, generally, the tissue and blood constituents are very specifically affected and altered consequent to the changes in their environment and the degree of infestation /infection. The use of blood and tissue chemistry as a diagnostic profile is not an uncommon practice, as such methods have already been proved to be valuable in human medicine and in animal breeding (both wild and domesticated animals) (Payne, 1985). Since, the disease patterns differ and the causes of the diseases are multifactorial, the results of such diagnostic profile tests could be correlated to the differing patterns of the disease. There for this investigation planed for evaluate the metabolic profile (biochemical and enzymatic) of *V. parahaemolyticus* MTCC 451 inoculated black tiger prawn *P. monodon* (Fabricius).

MATERIALS AND METHODS

Species collection: Specimens of *P. monodon* juvenile were collected from two commercial shrimp farms situated along the Chennai coastal area. Juvenile prawn ranging in size from 10-20 cm and weight from 15-20 g were selected and only the intermoult prawns were chosen for the experiments.

Microbial studies

Selection of bacterial inoculum: The bacterial strain, *Vibrio parahaemolyticus* MTCC 451 was selected as the biotoxin for the study. The bacterial strain, *Vibrio parahaemolyticus* MTCC 451 was bought from the Institute of Microbial Type Culture Collection and Gene Bank (IMTCC) Chandigarh, India.

Inoculums preparation and inoculation: The nutrient agar medium (MTCC growth medium 53) prepared and *Vibrio parahaemolyticus* MTCC 451 was inoculated. About 24 h culture was taken for the preparation of the inoculums. This was then diluted to two-fold serial dilutions of the bacterial suspension, which was made with different dilutions viz., 10^{-8} , 10^{-7} , 10^{-6} and 10^{-5} . About 0.05 mL of the inoculum was taken in 1 mL tuberculin syringe and injected in between the 5th and 6th abdominal segment. Care was taken to inject the whole inculum into the body for the determination of 96 h LD₅₀ value.

Bacterial count in the inoculums of *Vibrio parahaemolyticus* MTCC 451: The bacterial count or the colony forming units in the inoculums of LD₅₀ was determined by the procedure followed by Collee *et al.* (1996).

Toxicological studies

Acute toxicity test: Acute toxicity bioassay to determine the LD₅₀ dose of the inoculum was carried out by the modified Method described by Reed and Muench (1938). About 4 groups of prawns, each consisting of 10 prawns, were selected for acute toxicity studies. The prawns in each group were inoculated with bacterial suspension (*Vibrio parahaemolyticus* MTCC 451) at varying concentrations, viz 10⁻⁸, 10⁻⁷, 10⁻⁶ and 10⁻⁵. The cumulative percentage of mortality at the intervals of 24, 48, 72, 96 h was noted. The symptoms and behavioural changes were noted.

Enzyme activity studies: The levels of Alkaline phosphatase activity, Acid phosphatase activity, Lactate dehydrogenase activity, Glutamate Pyruvate Transaminase (GPT) activity, Glutamate Oxaloacetate Transaminase activity, Chitinase activity, Water content, Sodium, Potassium, Calcium, Ascorbic acid and Histamine in Haemolymph, Hepatopancreas and Body muscle of *P. monodon* control Vs tested were noted according o proper protocols.

Statistical analysis: Data from the present studies were subjected to standard deviation and the significance of the difference obtained was assessed by Two-way classification with replication type analysis of variance (ANOVA) for the comparison of control and experimental data.

RESULTS

The culture of *V. parahaemolyticus* MTCC451 in TCBS agar media. The culture of *V. parahaemolyticus* MTCC451 in nutrient agar media resulted in the growth of pale coloured colonies. In the TCBS agar media, the bacteria growth resulted as green colour colonies. The bacterial count or colony forming units of *V. parahaemolyticus* MTCC451 in the inoculated bacterial suspension with the known LD₅₀ value was determined to be 2.43×10⁷ CFU/0.05 mL of the inoculum (Table 1).

The injected *V. parahaemolyticus* MTCC451 resulted in the outbreak of vibriosis in the tested *P. monodon* with the exhibition of following clinical signs viz., translucent appearance of the abdominal musculature to a whitish opaque colouration; slight darkening of the dorsal portion of the integument; eroded localized lesion in the cuticle that is melainised (Brown to black) at later stages, anorexia, reddening of the periopods and pleopods; pale and atrophied hepatopancreas and off-fed empty guts. Table 2 shows the biochemical and enzyme studies on *P. monodon* control (0 h) Vs inoculated with *V. parahaemolyticus* MTCC 451.

In enzyme activity, the levels of alkaline phosphatase activity in the haemolymph, hepatopancreas and muscle of *P. monodon* control Vs tested are given in Fig. 1. The alkaline phosphatase activity in the haemolymph were significantly higher in treated animals at all intervals, except at 72 h, with respect to the control group (p = 0.002*) (p<0.05). At 24 h, it was 1/3rd the level observed in the control group raising gradually to approximately the level in the control until 72 h after, which it declined. The alkaline phosphatase activity in the haemolymph

Table 1: Lethal Dosage (LD₅₀) of *Vibrio parahaemolyticus* MTCC 451 in *Penaeus monodon*

No. of prawns challenged	Concentration of the inoculums	No. of prawns died in 24 h	No. of prawns died in 48 h	No. of prawns died in 72 h	No. of prawns died in 96 h	No. of prawns dead	Mortality (%)
10	10 ⁻⁵	2	2	1	3	8	80
	10 ⁻⁶	2	3	1	1	7	70
	10 ⁻⁷	1	2	2	-	5	50
	10 ⁻⁸	-	1	1	-	2	20

Bacterial count in the LD₅₀ value inoculums of 10⁻⁷ = 2.43 CFU/0.05 mL

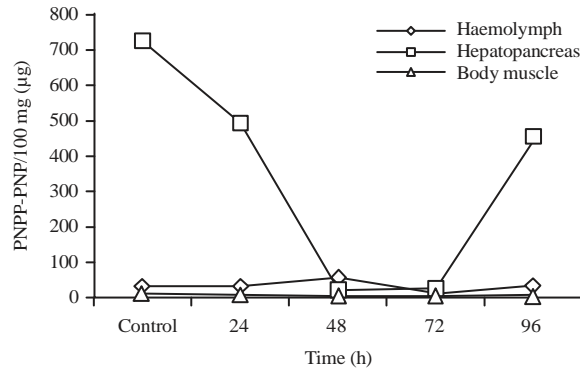


Fig. 1: Alkaline phosphatase in the haemolymph, hepatopancreas and body muscle of *P. monodon* control (0 h) vs inoculated with *V. parahaemolyticus* MTCC 451-A time course study

Table 2: Biochemical studies on *P. monodon* Control (0 h) vs inoculated with *Vibrio parahaemolyticus* MTCC 451-A time course study

Enzymatic studies	Prawn samples	Mean					p-value
		Control	24 h	48 h	72 h	96 h	
Alkaline phosphatase activity	Haemolymph	31.014	34.195	60.438	11.133	33.400	0.002*
	Hepatopancreas	720.480	494.760	24.890	24.970	456.860	1.20E-14*
	Body muscle	10.258	4.532	3.500	1.500	1.500	0.004*
Acid phosphatase activity	Haemolymph	0.760	12.750	3.061	7.140	11.600	6.77E-05*
	Hepatopancreas	16.190	46.200	1.350	2.840	9.270	5.56E-17*
	Body muscle	0.940	3.900	0.330	1.540	1.550	4.70E-05*
Lactate dehydrogenase activity	Haemolymph	0.031	0.049	0.019	0.007	0.021	1.94E-06*
	Hepatopancreas	0.008	0.044	0.022	0.028	0.023	2.92E-03*
Glutamate Pyruvate Transaminase (GPT) activity	Haemolymph	0.037	0.043	0.026	0.019	0.059	0.058
	Hepatopancreas	300.830	973.750	617.500	1425.000	1282.500	3.39E-06*
	Body muscle	74.410	41.960	7.500	21.770	34.040	0.006*
Glutamate oxaloacetate transaminase activity	Haemolymph	180.500	205.830	210.580	216.120	206.600	0.785
	Hepatopancreas	209.310	514.310	167.430	669.800	520.300	1.03E-08*
	Body muscle	59.200	3.290	7.770	27.510	63.400	1.96E-09*
Chitinase activity	Haemolymph	49.600	57.400	39.470	92.700	116.020	2.71E-09*
	Hepatopancreas	276.670	213.340	166.670	653.340	213.340	1.41E-08*
	Body muscle	46.000	48.340	69.000	39.670	35.670	0.243
Water content	Haemolymph	7.230	7.670	4.900	7.860	2.060	0.002*
	Hepatopancreas	77.670	83.330	81.000	81.330	78.670	0.542
	Body muscle	68.500	73.340	77.500	74.500	72.340	0.082
Sodium	Haemolymph	73.170	80.670	73.670	82.160	77.160	0.024*
	Hepatopancreas	31.450	58.136	36.530	44.208	14.940	0.012*
	Body muscle	21.610	21.200	23.400	28.350	5.096	0.173
Potassium	Haemolymph	35.400	28.200	32.260	23.870	23.940	0.513
	Hepatopancreas	158.560	130.000	127.000	165.300	618.750	1.96E-13*
	Body muscle	106.250	150.000	131.250	119.370	115.625	0.012*
Calcium	Haemolymph	146.870	137.500	171.870	159.370	143.750	0.227
	Hepatopancreas	455.000	225.000	413.750	375.000	500.000	0.0006*
	Body muscle	137.500	175.000	125.000	100.000	250.000	0.125
Ascorbic acid	Haemolymph	125.000	112.500	162.500	137.500	162.500	0.703
	Hepatopancreas	1127.460	775.900	1075.600	481.880	442.740	5.80E-24*
	Body muscle	337.040	54.890	53.235	33.560	326.940	1.89E-19*
Histamine	Haemolymph	312.300	282.570	224.130	207.230	317.750	2.19E-07*
	Hepatopancreas	31.452	58.136	36.530	44.210	14.940	3.62E-12*
	Body muscle	21.610	21.200	23.400	28.350	5.100	1.52E-10*
		35.400	28.200	32.260	23.870	23.900	0.0005*

*Significant at 0.05% level (p<0.05)

were significantly lower in treated animals at all intervals with respect to control (p = 0.0001*) (p<0.05). The levels of alkaline phosphatase activity in the hepatopancreas was significantly lower

in treated animals at all intervals with respect to the control group ($p = 1.20E-14^*$) ($p < 0.05$). The alkaline phosphatase activity in the body muscle showed a significant lower in treated animals at all intervals with respect to control group ($p = 0.004^*$) ($p < 0.05$).

The levels of acid phosphatase activity in the haemolymph, hepatopancreas and muscle of *P. monodon* control vs tested are given in Fig. 2. The acid phosphatase activity in the haemolymph was significantly higher in treated animals at all intervals with respect to the control group ($p = 6.77E05^*$) ($p < 0.05$). The acid phosphatase activity in the haemolymph was significantly lower in treated animals at all intervals except 24 h, with respect to control. At 24 h, the enzyme activity was approximately three-fold greater the level observed in the control group ($p = 5.56E-17^*$) ($p < 0.05$). The acid phosphatase activity in the body muscle was significant higher in treated animals at all intervals except at 48 h, with respect to control group ($p = 4.70E-05^*$) ($p < 0.05$). At 48 h, it showed a gradual decrease and increased at 72 and 96 h of inoculation.

The levels of lactate dehydrogenase activity in the haemolymph, hepatopancreas and muscle of *P. monodon* control Vs tested are given in Fig. 3. The lactate dehydrogenase activity in the haemolymph was significantly lower in treated animals at all intervals, except at 24 h, with respect to the control group ($p = 1.94E-06^*$) ($p < 0.05$). The lactate dehydrogenase activity in the haemolymph was significantly higher in treated animals at all intervals with respect to control

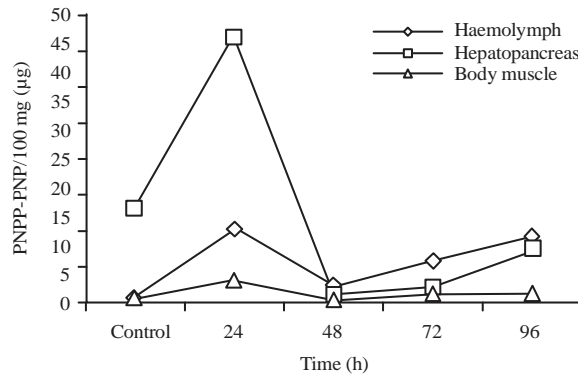


Fig. 2: Acid phosphatase in the haemolymph, hepatopancreas and body muscle of *P. monodon* control (0 h) vs inoculated with *V. parahaemolyticus* MTCC 451- A time course study

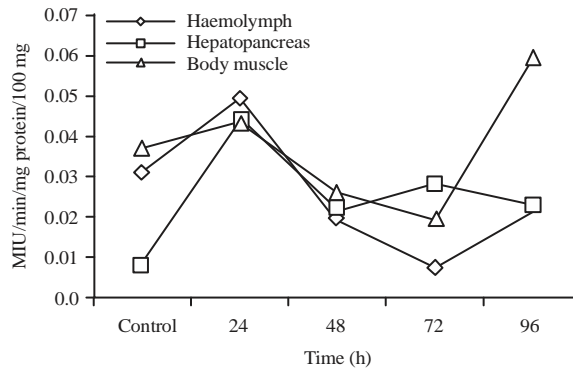


Fig. 3: Lactate dehydrogenase activity in the haemolymph, hepatopancreas and body muscle of *P. monodon* control (0 h) vs inoculated with *V. parahaemolyticus* MTCC 451-A time course study

($p = 2.92E-03^*$) ($p < 0.05$). The lactate dehydrogenase activity in the body muscle was insignificant higher in treated animals at certain levels with respect to the control group ($p = 0.058$) ($p > 0.05$). At 24 h, the activity increased and then gradually decreased after 48 and 72 h, after, which it increased at 96 h.

The levels of Glutamate Pyruvate Transaminase (GPT) activity in the haemolymph, hepatopancreas and muscle of *P. monodon* control Vs tested are given in Fig. 4. The Glutamate Pyruvate Transaminase activity in the haemolymph was significantly higher in treated animals at all intervals with respect to the control group ($p = 3.39E-06^*$) ($p < 0.05$). At 24 h, the enzyme activity increased at almost three-fold the level compared to that of control group. It then decreased at 48 h and increased at 72 and 96 h of inoculation. The Glutamate Pyruvate Transaminase activity in the body muscle was insignificant higher in treated animals at all intervals with respect to the control group ($p = 0.785$) ($p > 0.05$). The enzyme activity gradually increased at the intervals of 24, 48 and 72 h after which it declined at 96 h.

The levels of Glutamate Oxaloacetate Transaminase activity in the haemolymph, hepatopancreas and muscle of *P. monodon* control Vs tested are given in Fig. 5. The glutamate oxaloacetate transaminase activity in the haemolymph was significantly higher in treated animals at all intervals, except at 48 h, with respect to the control group ($p = 1.03E-08^*$) ($p < 0.05$). At 24 h,

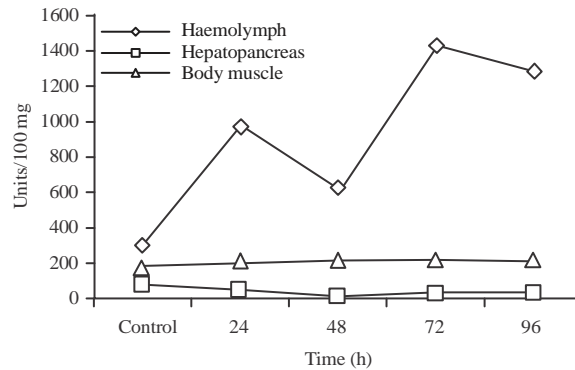


Fig. 4: Glutamate pyruvate transaminase activity in the haemolymph, hepatopancreas and body muscle of *P. monodon* control (0 h) Vs inoculated with *V. parahaemolyticus* MTCC 451- A time course study

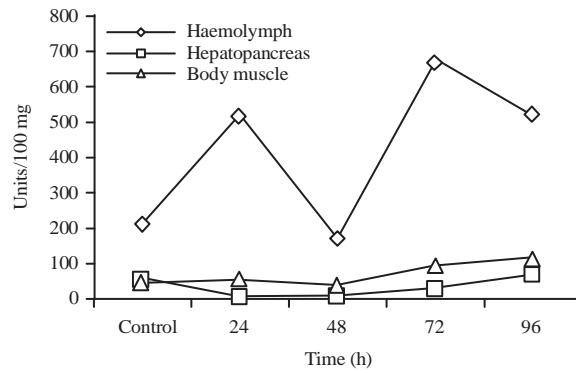


Fig. 5: Glutamate Oxaloacetate transaminase activity in the haemolymph, hepatopancreas and body muscle of *P. monodon* control (0 h) Vs inoculated with *V. parahaemolyticus* MTCC 451 -A time course study

it was two-fold higher the level observed in the control group and decreased at 48 h. It was followed by an increase and decrease at 72 and 96 h, respectively. The glutamate oxaloacetate transaminase activity in the hepatopancreas was significantly higher in treated animals with respect to control ($p = 1.03E-08^*$) ($p < 0.05$). At 24 h, the enzyme activity decreased markedly but it increased after 48, 72 and 96 h of inoculation. The glutamate oxaloacetate transaminase activity in the body muscle was significant higher in treated animals at all intervals except at 48 h, with respect to the control group ($p = -1.03E-08^*$) ($p < 0.05$).

The levels of chitinase activity in the haemolymph, hepatopancreas and muscle of *P. monodon* control vs tested are given in Fig. 6. The chitinase activity in the haemolymph was significantly lower in treated animals at all intervals, except at 72 h, with respect to the control group ($p = 1.41E-08^*$) ($p < 0.05$). At 72 h, it increased approximately to three-fold the level observed in the control group. The chitinase activity in the hepatopancreas was insignificantly lower in treated animals at some intervals with respect to control ($p = 0.243$) ($p > 0.05$). The enzyme activity was gradually increased at 24 and 48 h and then gradually decreased at the intervals of 72 and 96 h in the experimentation. The chitinase activity in the body muscle was significantly lower in treated animals at certain intervals with respect to the control group ($p = 0.002^*$) ($p < 0.05$). The enzyme activity increased slightly at 24 h and decreased at 48 h and then again an increase and decrease at 72 and 96 h, respectively.

The levels of water content in the haemolymph, hepatopancreas and muscle of *P. monodon* control vs tested are given in Fig. 7. The water content in the haemolymph was insignificantly higher in treated animals at all intervals with respect to the control group ($p = 0.542$) ($p > 0.05$). The water content increased throughout the experimentation to that of the zero hrs control level. The water content in the hepatopancreas was insignificantly higher in treated animals at all intervals with respect to the control group ($p = 0.082$) ($p > 0.05$). The water content increased throughout the experimentation to that of the control level. The water content in the body muscle was significantly higher in treated animals at all intervals with respect to the control group ($p = 0.024^*$) ($p < 0.05$). The water content in the body muscle increased throughout the experimentation to that of the zero hrs control level.

The levels of sodium in the haemolymph, hepatopancreas and muscle of *P. monodon* control vs tested are given in Fig. 8. The sodium level in the haemolymph was significantly lower in treated animals at all intervals respect to the control group ($p = 0.012^*$) ($p < 0.05$). The sodium level in the hepatopancreas was insignificantly higher in treated animals at all intervals with respect to control

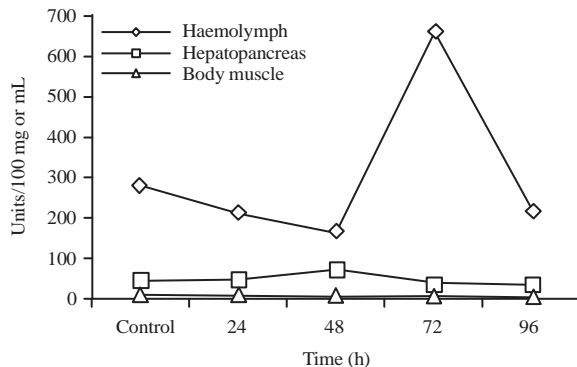


Fig. 6: Chitinase activity in the haemolymph, hepatopancreas and body muscle of *P. monodon* control (0 h) vs inoculated with *V. parahaemolyticus* MTCC 451-A time course study

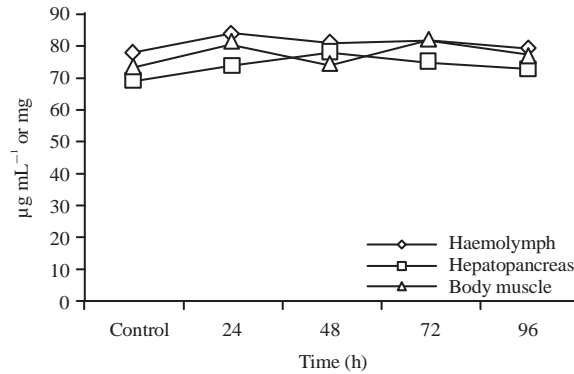


Fig. 7: Water content in the haemolymph, hepatopancreas and body muscle of *P. monodon* control (0 h) vs inoculated with *V. parahaemolyticus* MTCC 451-A time course study

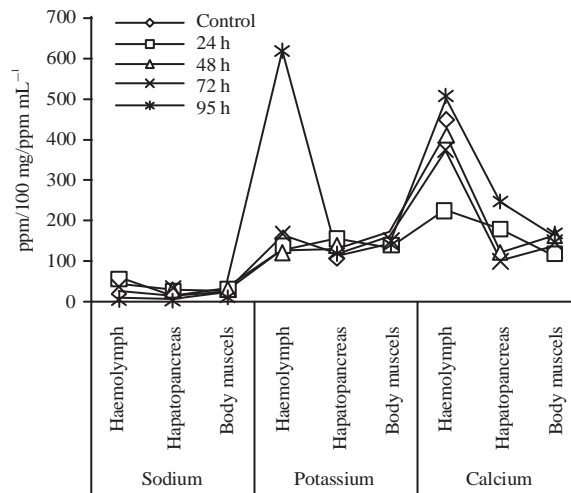


Fig. 8: Sodium, Potassium and Calcium levels in the haemolymph, hepatopancreas and body muscle of *P. monodon* control (0 h) vs inoculated with *V. parahaemolyticus* MTCC 451-A time course study

($p = 0.173$) ($p > 0.05$). The sodium level in the body muscle was insignificant higher in treated animals at all intervals except at 72 h, with respect to the control group ($p = 0.513$) ($p > 0.05$).

The levels of potassium in the haemolymph, hepatopancreas and muscle of *P. monodon* control Vs tested are given in Fig. 8. The potassium level in the haemolymph was significantly higher in treated animals at all intervals, except to 48 h, with respect to the control group ($p = 1.96E-13^*$) ($p < 0.05$). it decreased after 24 and 48 h to that of zero hrs control level but increased after 72 and 96 h. The potassium level in the hepatopancreas was significantly higher in treated animals at all intervals with respect to control ($p = 0.012^*$) ($p < 0.05$). The potassium level in the body muscle was insignificant higher in treated animals at certain intervals with respect to the control group ($p = 0.703$) ($p > 0.05$). The level decreased after 24 h but increased after 48 h. It showed a decrease after 72 and 96 h of inculcation.

The levels of calcium in the haemolymph, hepatopancreas and muscle of *P. monodon* control vs tested are given in Fig. 8. The calcium level in the haemolymph was significantly lower in

treated animals at 96 h interval with respect to the control group ($p = 0.0006^*$) ($p < 0.05$). It decreased after 24 h to that of zero hrs control level but elevated after 48 h. It decreased after 72 h and increased at 96 h. The calcium level in the hepatopancreas was insignificantly higher in treated animals at certain intervals with respect to the control ($p = 0.125$) ($p > 0.05$). It increased after 24 h to that of zero h control level but decreased after 48 and 72 h. It increased at 96 h in the experimentation. The calcium level in the body muscle was insignificantly higher in treated animals at certain intervals with respect to the control group ($p = 0.513$) ($p > 0.05$). It increased after 24 and 48 h. It decreased after 72 h, followed by an increase at 96 h.

The levels of ascorbic acid in the haemolymph, hepatopancreas and muscle of *P. monodon* control vs tested are given in Fig. 9. The ascorbic acid level in the haemolymph was significantly lower in treated animals at 96 h all intervals with respect to the control group ($p = 5.80E-19^*$) ($p < 0.05$). The ascorbic acid level in the hepatopancreas was significantly lower in treated animals at all intervals with respect to the control ($p = 1.89E-19^*$) ($p < 0.05$). The ascorbic acid level in the body muscle was significantly higher in treated animals at 96 h intervals with respect to the control group ($p = 2.19E-07^*$) ($p < 0.05$). The level decreased after 24, 48 and 72 h to that of control level. It increased at 96 h in the experimentation.

The levels of histamine in the haemolymph, hepatopancreas and muscle of *P. monodon* control Vs tested are given in Fig. 10. The histamine level in the haemolymph was significantly lower in

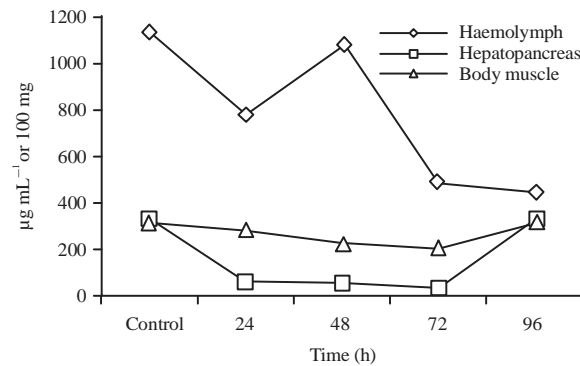


Fig. 9: Ascorbic acid levels in the haemolymph, hepatopancreas and body muscle of *P. monodon* control (0 h) vs inoculated with *V. parahaemolyticus* MTCC 451 - A time course study

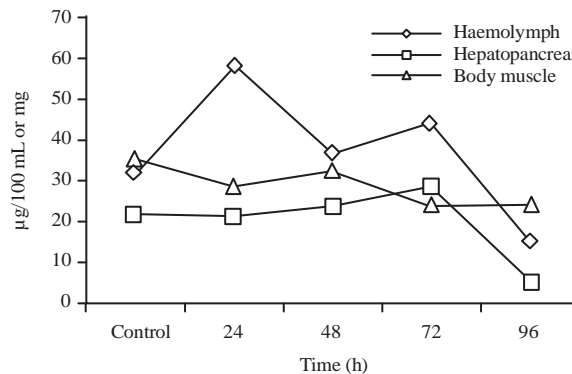


Fig. 10: Histamine levels in the haemolymph, hepatopancreas and body muscle of *P. monodon* control (0 h) vs inoculated with *V. parahaemolyticus* MTCC 451 - A time course study

treated animals at 96 h all intervals with respect to the control group ($p = 3.62E-12^*$) ($p < 0.05$). The level showed an increase after 24 h but decreased after 48 h and till 96 h. The histamine level in the hepatopancreas was significantly lower in treated animals at certain intervals with respect to the control ($p = 1.52E-10^*$) ($p < 0.05$). The level showed a decrease after 24 h. It increased after 48 and 72 h followed by a significant decrease after 96 h. The histamine level in the body muscle was significantly lower in treated animals at all intervals with respect to the control group ($p = 0.0005^*$) ($p < 0.05$).

DISCUSSION

In normal, diseases in aquaculture practices are mostly caused by *Vibrio* sp and it has been referred as the largest economic loss in the shrimp aquaculture due to mass mortalities (Karthik *et al.*, 2014). In the present study, *P. monodon* inoculated with *V. parahaemolyticus* MTCC451, exhibits clinical signs, which include; translucent appearance of the abdominal musculature and whitish opaque coloration; slight darkening of the dorsal portion of the integument; eroded localized lesions in the cuticle, which subsequently melanise (brown to black); reddening of the periopods and pleopods; pale and atrophied hepatopancreas and empty guts etc.

Bechteler and Holler (1995) revealed the clinical signs of Vibriosis in shrimps viz., loss of appetite, a halt in growth, broken antennae, necrosis, lesions and black spots in the muscle tissue. Bowers *et al.* (1996) reported the clinical signs of black discolouration of the cuticle especially around the edges of body segments and black stippled on the surface of the hepatopancreas in the "Stained prawns disease" of *Pandalus platycerus* caused by rickettsial infection. In the present study, the darkening of the dorsal portion of the integument might be due to the microbial action of *V. parahaemolyticus* MTCC451, which possesses chitinase, oxidase and catalase enzymes. The phenoloxidase enzyme of the *P. monodon* might have produced the blacking (melanin formation) of the infected cuticle. The erosion and destruction of the cuticle might be due to the action of chitinase and proteolytic enzymes of the inoculated *Vibrio* species, leading to the breakdown of tissue and thus, possibly the loss of haemolymph and invasion of external pathogens. The localized lesion might be due to the accumulation of *Vibrio* in the lesion and the empty gut might be due to loss of appetite in the host (Lightner, 1993).

Comparable studies are meagre in invertebrates like crustaceans which are important cultivable species and prone to infections in the aquaculture system. Previous studies on crustacean comprise certain stress factors like autotomy, bleeding stress, transplantation, eye ablation etc. These studies have emphasized the impact of stressors on the homeostatic mechanisms in the blood and tissues involving the metabolites namely, carbohydrates, proteins and lipids. The haemocytes of crustaceans and other invertebrates play an important and central role in immune response, performing functions such as phagocytosis, encapsulation, nodule formation and mediation of cytotoxicity.

Freshwater prawns are less stressed by changes in the ambient conditions with regard to their marine counterparts. As the water and electrolyte status may serve as a sensitive physiological index, the ionic changes occurring in the tissues of the freshwater prawn, *Macrobrachium rosenbergii* (Ramalingam *et al.*, 2015). In the present study, the results revealed the total carbohydrate content showing a significant decrease after 96 h in all the three tissue viz., haemolymph, hepatopancreas and body muscle of prawns inoculated with *Vibrio parahaemolyticus* MTCC 451, when compared to that zero hour control value. Ramalingam and Ramarani (2006), revealed the sparing role of proteins next to carbohydrates in meeting the energy demand and they

reported that the same inference could not be attributed in the case of prawns subjected to *Pseudomonas aeruginosa* infection of their endotoxin toxicity. Recent studies have revealed that bacterial species can synthesis extra cellular proteases.

The shrimps are rich in protein content and hence they do not have a protein requirement but do have a requirement of amino acids. Protein plays a key role in the development growth and immunity of prawns. In the present study, the result demonstrate a significant decrease in the level of total proteins after 96 h in all the tissues viz., haemolymph, hepatopancreas and body muscle at all intervals of study. The decrease was found to be more pronounced in the hepatopancreas, when compared to that of haemolymph and body muscle, thus reveling that changes in total proteins differ among the various tissues. In hepatopancreas, there was constant decrease from that of control level after 24, 48, 72 and 96 h was also found to be lower to control level. In body muscle, the proteins decreased throughout the course of experimentation but in haemolymph the total proteins showed transient changes at all intervals with a peak level after 72 h, which was greater than the control level. Ramalingam and Ramarani (2006), stated that, proteases of bacterial origin may be said to degrade the tissue proteins in the prawns. The marked changes noticed in both muscle, hepatopancreas and the histopathological symptoms may also be attributed to the above bacterial proteases. When proteases of such bacterial origin attack the tissues, the histamine bound to the cell protein could have been released. In the microbial infections histamines are of importance, because they are attributed to bring deleterious effects of tissue inflammation.

Phosphatases are known to play an important role in acute energy crisis (Shymala, 2001). They serve as makers for the evaluation of disease or pathological conditions. Alkaline phosphatase is a brush order enzyme, which splits various phosphorous esters at an alkaline pH and mediates membrane transport. Alkaline phosphatase has been reported to be of metabolic significance and plays an important role in several metabolic processes. Acid phosphatase is a loysosomal enzyme, which hydrolyzes phosphorous esters in an acid medium. These enzymes are non-specific in their site of action as well as to substrates. The enhancement of esterase enzymes like acid phosphatase and alkaline phosphatase is quite conceivable.

The laboratory results of the present investigation, using the inoculum *Vibrio parahaemolyticus* MTCC451 revealed that these endemic bacterial populations might become opportunistic pathogens, when hydroecological conditions are altered in the farm ponds and could bring the fatal episodes of mass mortality.

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