EFFECT OF VIBRIO *SPECIES* ASSOCIATED WITH HAEMOLYMPH IN THE CRAB, *PARATELPHUSA JACQUEMONTII*

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Abstract: The present research paper deals with effects of Vibrio species on Haemolymph of Crab Paratelphusa jacquemontii(Rathban),was observed during laboratory experimental conditions. The results were recorded that, infection of Vibrio sps.in haemolymph alters Total leucocyte count (TLC) and Differential leucocyte count (DLC)as wel as augmentation of disease in the body of Crab, P. jacquemontii (Rathban).Water pollution also affects health of said Crab.Due to this contamination of crabs with microorganism happens,the microorganisms are potential pathogens of human beings. Hence the research study suggest that,contaminated Crabs are never used as a Food. Peoples shall use uncontaminated Crabs as a Food.

Keywords: **Effects of Vibrio Species on edible Crab, Paratelphusa jacquemontii.**

Introduction: India is a country having great cultural diversity associated with all kinds of climates, rich flora and fauna, and supporting an estimated total of eight percentages of the globally documented species. It is experiencing increasing pressure on its bioresource and ecosystem services due to high demand of food **(Kannupandi *et al.*, 2003; Varadharajan *et al.*, 2009)**. Microorganisms are widely distributed in nature and diversity of microorganisms may be used as an indicator for organic pollution. The bacteria are basically suffocating the other organisms. Some of the organisms that overpopulate from this can also are disease-causing microorganisms. Increasing pollution of rivers and other water bodies has become a matter of great concern in recent years. Further crabs, fishes are the non-target organisms are affected. The accumulated toxicant in their body may reach the consumers.

These variations are often stressing crustacean, resulting in a reduction of immune strength. Water quality monitoring has one of the highest significances in environmental protection policy. The main objective is to control and minimize the incidence of pollutant oriented problems, and to provide water of good quality to serve various environmental purposes **(Sargaonkar and Deshpande, 2003)**. The water quality is also affected by pollutants which act on elements existing in water such as dissolved oxygen or produce substances such as ammonia, nitrates etc. It is not possible to understand biological phenomena fully without the knowledge of water chemistry. If we can find some correlations among these numerous parameters, however, the task of periodic monitoring of water quality may be facilitated to a good extent **(Tiwari, 2011)**. Crustacean‟s hemocytes are known for their importance in defense reactions towards invasive microorganisms infection by several species of bacteria has been documented in decapods occupying freshwater habitats **(Johansson *et al.*, 2000).**
Haemocyte classification in various crustaceans lacks consistency. Classification of the haemocyte types in decapod crustaceans is based mainly on the presence of
cytoplasmic granules in hyaline cells, semi-granular cells and granular cells
(Persson *et al.*, 1987). The same classification was given by **(Johansson *et al.*, 1989)** for crayfish. The circulating haemocyte number is a stress indicator **(Le**
Moullac *et al.*, 2000) and haemocyte counts may be a valuable tool in monitoring the health status of crustacean species. The haematological index has been employed effectively in monitoring the response of the aquatic animals to the stressors and thus its health status under such adverse conditions and reflects the ecological condition of its habitat.

Material and Methods:

The fresh water crabs of species *Paratelphusa jacquemontii* were maintained in the laboratory in well water collected and exposed to bacterial and fungal inoculums to study the haematological and histopathological changes due to the stress induced by the microbes. **Selection of Bacteria for experiment:**
Vibrio sp. isolated from the water samples of Nal-Damyanti Sagar dam were selected as test micro-organisms for the experimental study. *Vibrio sp.* was selected considering their ubiquitous presence in the aquatic environment and their role as an important crab pathogen in fresh water crabs. This micro-organism was selected hypothesizing that in a stressed environment the different bacterial species could negatively affect the crab fauna
Collection: Crabs were captured manually using dip net with the help fishermen (4-5 individuals). Crabs
Experimental and control individuals check over a period of 15 days recording the changes of the THC and DHC were counting to initially and at 2nd, 4th, 6th, 8th,10th,12th and later on at 15th day

Haematological analysis

Collection of heamolymph: For the study of haemocytes and their differential count, the haemolymph of *P. jacquemontii* was collected aseptically from the base of one of the second walking legs **(Mattson and Spaziani, 1985; Eddy *et al.*, 2007)** using a 1ml sterile syringe, place in Eppendorf tubes and diluted 1:2 in an anticoagulant solution with ice-cold citrate EDTA buffer (0.45 M NaCl; 0.1 M glucose; 30 mM trisodium citrate; 20 mM citric acid; 100
mM EDTA, pH 4.6).

Total haemocyte count (THC):

Total haemocyte counts (THC) were made using Neubauer chamber and a procedure similar to that used for red blood counts. Each experiment was repeated three times **(Eddy *et al.*, 2007; Manjula *et al*., 1997).**

Differential haemocyte count (DHC):

Differential haemocyte counts (DHC) were estimated according to the method of **Nakayama *et al.*, (1997)** using Giemsa stain. A sample of 100µl of haemolymph was taken on a clean, grease- free glass slide and allowed to form a monolayer on the slide by incubating in a moist chamber for 45 minutes. It was fixed with 10% Methanol for 15min, air dried, and stained in Giemsa for 20 min. The slides were then viewed under a research microscope to identify haemocytes. For differential count, a total of 200 cellswere counted and the percentage of each type of cells such as granulocyte, semigranulocytes, and hyalinoctes was calculated.

Table: Response of THC in *Paratelphusa jacquemontii* exposed to *Vibrio sp.*

- Indicates mortality

Bacterial Dose	**THC (Cell/mm3)**						
	Day2	**Day 4**	**Day 6**	**Day8**	**Day10**	**Day 12**	**Day15**
0 (Control)	5028 ± 7.63	4990± 20	5106 ± 51.31	5086 ± 50.47	5040 ± 140.0	5030± 49.58	5046± 50.52
10^3 (High)	1530 ±26.45	1311 ± 35.47	-	-	-	-	-
10^5 (Medium)	2046± 15.27	1823 ± 16.07	1681± 10.40	1463± 47.82	-	-	-
10^7 (Low)	3816± 25.16	3962 ± 54.04	4271± 17.55	4561 ± 39.71	4990± 28.67	4980± 280.08	4950± 26.12

Table : Response of DHC in *Paratelphusa jacquemontii* exposed to *Vibrio sp.*

Day	Dose	DHC (%)		
		Granulocyte	Semigranulocyte	Hylinocytes
2	10^3	63	25.45	8.33
	10^5	53.03	22.33	11.66
	10^7	52.01	29.66	15.4
4	10^3	53.66	21	10.33
	10^5	55	29.33	12.66
	10^7	57	24	13.4
6	10^3	-	-	-
	10^5	52.2	32.2	16.1
	10^7	51.03	32.24	14.2
8	10^3	-	-	-
	10^5	55.33	29.66	15
	10^7	58	30	14.33
10	10^3	-	-	-
	10^5	-	-	-
	10^7	53.43	30.2	16.66
12	10^3	-	-	-
	10^5	-	-	-
	10^7	52.01	29.66	15.4
15	10^3	-	-	-
	10^5	-	-	-
	10^7	52.2	32.2	16.1

- Indicates mortality

Result:-

Haemocytes pathology:
Impact of *Vibrio sp.*

Total Haemocyte Count (Control): The count is 5028 ± 7.63 at 2nd day and 5046 ± 50.52 at 15th day.

Total Haemocyte Count (10^3): The count of THC by exposure of high dose of *Vibrio sp.* is 1530 ± 26.45 at 2nd day and 1311 ± 35.47 at 4th day. Mortality occurs at 6th day.

Total Haemocyte Count (10^5): The count of THC by exposure of medium dose is 2046 ± 15.27 at 2nd day and 1463 ± 47.82 at 8th day. Mortality occurs at 10th day.

Total Haemocyte Count (10^7): The count of THC by exposure of low dose is 3816 ± 25.16 at 2nd day and 4950 ± 26.12 at 15th day. (Table- 4.20).
Vibrio sp. the gram negative pathogen (1ml) was injected through the walking legs to assess the pathological impact. It was found lethal to *P. jacquemontii* within short period. In 103 dilutions there was a significant fall in the number of circulating haemocytes on Day-2 (1530 ± 26.45 cells / mm3) and subsequently there was depletion in the haemocytes number on Day-4 (1311 ± 35.47 cells/ mm3). Mortality occurs at Day-6. Similar observation was recorded in 105 dilutions.
Mortality occurs on 10th day. But in 107 dilutions even though there was a decline in haemocyte count initially, there was a gradual increase in the number of circulating haemocytes (3816 ± 25.16 to 4950 ± 26.12 cells / mm3).

Discussion

Experimental infection of bacterial strain i.e. *Vibrio sp*., *E coli*. and Fungal strain *Aspergillus sp*. show fluctuation in haemolymph parameters. Experimental infection of bacterial sp. results in to rapid decrease in total haemocyte count. In high dose (103) there was a significant fall in the circulating haemocyte result in to early mortality. In low dose (107) though there was a decline in haemocyte initially but then there was gradual increase in the haemocyte count. In the present study, *vibrio sp.* and *E. coli*. show profound influence on the population of granulocyte and Semigranulocyte ratio. The present investigation showed that the high bacterial and fungal load in water, so crab also infect by isolated bacteria
The consumption of these disease or infected crabs possesses greater health risks than the consumption of apparently healthy ones. The general public and consumers of crabs should therefore ensure that they do not buy or consume disease or injured crabs. Organic materials of any type are suitable foodstuffs for bacteria growth. A case of cholera occurred in a patient in Maryland, who had eaten crabharvested commercially along the Texas coast in October 1984. Findings of *Vibrio sp.* in the tissue of crabs of present studies are considered to be correlated with the epidemiology and transmission of cholera in the aquatic environment.

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