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RESEARCH ARTICLE

STUDIES ON THE OCCURRENCE OF *VIBRIO SP.* WITH REFERENCE TO ENVIRONMENTAL FACTORS OF SHRIMP CULTURE POND OF OLPAD REGION IN SURAT

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Abstract

Physico-chemical and microbiological analyses of pond water was conducted to determine if there is any relation between the occurrence of *Vibrio sp.* and aquatic environmental factors in shrimp culture pond, Olpad, Surat city. Water samples were analysed for different environmental factors such as temperature, pH, dissolved oxygen, salinity, ammonia and hydrogen sulphide. Microbiological study in reference to *vibrio sp.* was also done by membrane filter technique. Among these water parameters alkaline pH, dissolved oxygen, appropriate temperature and salinity required for shrimp growth. Along with this parameters ammonia, hydrogen sulphide and salinity favours the occurrence of *Vibrio cholerae* and *Vibrio parahaemolyticus*. These highly salt tolerant bacteria may cause health hazard to human. Therefore this Study recommended to develop an antibacterial drug to control *Vibrio sp.* in shrimp aquaculture system to maintain good environment.

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Introduction:-

Shrimp farming has become an important economic activity in many developing countries (FAO, 2006). Recently, an increasing number of shrimp farms have upraised concern about water quality and shrimp health because of their economic impact on farm management. *Vibrio sp.* are one of the most commonly-occurring bacteria during shrimp farming (Vandenbergh et al., 2003). Most farmers now recognize the relevance of *Vibrio sp.* and their safety levels in shrimp ponds as *Vibrio sp.* are commonly used as environmental health indicators. The routine determination of *Vibrio sp.* in farms is quite useful in terms of successful pond management.

The number of reported *Vibrio sp.* has increased rapidly in the last decade. Thompson et al., (2004a) have reported 63 species comprising the genus *Vibrio*. Ten of them are of human concern (Twedt, 1989) since they have been associated with skin infections and severe gastrointestinal disorders (Andrews, 2004). Among more than 20 *Vibrio sp.* known to be associated with human disease, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* are most important. Ottaviani et al., (2005) and Austin (2010) reported *Vibrio cholerae*, *Vibrio vulnificus*, *Vibrio parahaemolyticus* and more recently *Vibrio mimicus* have been associated with gastrointestinal and skin infections, as well as septicaemia. Many seafood associated disease outbreaks have been reported worldwide (Nascimento et al., 2001; Morris, 2003). Some of the pathogenic *Vibrio sp.* have also been reported as the causal agents of shrimp infections (Goarant et al., 1999). Some of the *Vibrio sp.* such as *Vibrio harveyi* and *Vibrio parahaemolyticus* are also associated with bacterial infections in shrimp (Jiravanichpaisal and Miyazaki, 1995; Lavilla-Pitogo, 1995) and are generally considered to be opportunistic pathogens causing disease when shrimp are stressed.

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Shrimp ponds are stressful environments compared to estuaries or other enclosed water bodies (Direkbusararam et al., 1998). This is mainly due to high organic matter and dissolved oxygen fluctuations which affect the composition of natural bacterial communities. Under normal conditions, temperature increments will also bring about a greater diversity of *Vibrio sp.* (Pfeffer et al., 2003). When high temperatures and high salinity conditions prevail, some species such as *Vibrio parahaemolyticus* will predominate (Williams and LaRock, 1985).

The objective of this study was to check the occurrence of *Vibrio sp.* with reference to different physico-chemical parameters of shrimp culture pond located in Olpad region of Surat.

Materials and Methods:-

Water samples were collected from shrimp culture pond situated at Hathisa village, Olpad taluka of Surat city in Gujarat, India. Samples were collected twice in sterile screw-capped bottles for water and microbiological analyses under aseptic conditions. The Sterile bottles were brought to the lab in thermocoal box inside covered with ice pack.

Samples were analyzed for the physico-chemical parameters such as temperature, pH, dissolved oxygen, salinity, ammonia and hydrogen sulphide as described in Trivedi and Goel (1986). The temperature measurement and fixation of dissolved oxygen were done at site itself.

Simultaneously samples were analyzed for microbiological study by using membrane filter technique given by APHA (2005). Selective media (Thiosulphate citrate bile salts sucrose agar) and different biochemical media were used for identification. The organisms were identified by cultural, morphological and biochemical characteristics.

Results:-

Results of physico-chemical parameters and microbiological study are depicted in Table I.

Table-I:- Physico-chemical and Microbiological analyses of pond water.

Sr.No.	Parameter	Sample-I	Sample-II
Physico-chemical analyses			
1.	Temperature (°C)	24.6	23.1
2.	pH	8.06	8.56
3.	Dissolved oxygen (mg/l)	8.8	4.27
4.	Salinity (ppt)	25.2	25.6
5.	Ammonia (mg/l)	3.5	6.3
6.	Hydrogen sulphide (mg/l)	0.85	1.36
Microbiological analysis			
1.	Membrane filter technique	62 isolates/100 ml (<i>Vibrio cholerae</i> , <i>Vibrio parahaemolyticus</i>)	88 isolates/100 ml (<i>Vibrio cholerae</i> , <i>Vibrio parahaemolyticus</i>)

Temperature was recorded 24.6°C during first sampling while 23.1°C during second sampling. pH was found alkaline 8.06 and 8.56 respectively during both sampling period. During first sampling dissolved oxygen was observed 8.8 mg/l while it was observed very low 4.27 mg/l during end of the sampling. Salinity was found higher 25.2 ppt and 25.6 ppt respectively during each sampling period. Ammonia level increased from 3.5 mg/l to 6.3 mg/l respectively during sampling period. Hydrogen sulphide was recorded 0.85 mg/l during first sampling while it was found 1.36 mg/l during second sampling.

In microbiological analyses, 62 isolates/100 ml were recorded from first sampling while it increased i.e 88 isolates/100 ml during second sampling. *Vibrio cholerae* and *Vibrio parahaemolyticus* were found in both of the samples.

Vibrio cholerae seen yellow in color on Thiosulphate citrate bile salts sucrose (TCBS) agar. *Vibrio cholerae* exhibited positive reactions to motility and negative to gram stain. *Vibrio cholerae* was found to be Indole, Citrate utilization, Gelatin liquification, nitrate reduction and oxidase test positive while methyl red was negative.

Organisms fermented glucose, sucrose, maltose and mannitol. Above confirmed results of selective media, gram staining and biochemical tests indicated confirmed presence of *Vibrio cholerae* in water samples.

Vibrio parahaemolyticus seen green in color on TCBS agar. *Vibrio parahaemolyticus* exhibited positive reactions to motility and negative to gram stain. *Vibrio parahaemolyticus* was found to be Indole, Citrate utilization, Gelatin liquification and oxidase test positive. Organisms fermented glucose and mannitol. Above confirmed results of selective media, gram staining and biochemical tests indicated confirmed presence of *Vibrio parahaemolyticus* in water samples.

Discussion:-

The effect of physicochemical factors has been widely studied for pathogenic *Vibrio sp.*, especially for *Vibrio cholerae*. The potential significance of environmental factors on the dynamics of the disease caused by *Vibrio cholerae* has been reported in several studies, with temperature and salinity being the major factors affecting its ecology (Pardio-Sedas, 2007). Generally, *Vibrio sp.* are more common in warm waters, particularly when the water temperature is higher than 17°C (Thompson et al., 2004b). Findings of present study also showed that *Vibrio cholerae* and *Vibrio parahaemolyticus* were observed in higher temperature. Many researchers reported the abundance of *Vibrio parahaemolyticus* during summer in temperate zone, when temperature was above 25°C (Khan et al., 2002). Deepanjali et al., (2005) observed high levels of *Vibrio parahaemolyticus* during the dry season between January and May and decreased during post monsoon months. In tropical countries the seasonal cycle of the organism is correlated with rainy and dry seasons, the lowest numbers are found in rainy months, and the highest numbers are found in the dry season (Nelapati et al., 2012). Results of current work indicated that *Vibrio sp.* mainly affected by temperature.

Current study presented that dissolved oxygen was drastically dropped from first sampling to the end of the sampling period, but the growth of *Vibrio sp.* was reported continuously increasing. Results of present study were supported by Noriega-Orozco et al., (2007) showing *Vibrio sp.* capable of adjust to very low oxygen levels.

Present study revealed that the salinity levels of the study pond was near to recommended range 15-25 ppt (Boyd, 1995) but the number of *Vibrio* was increased in both samples within the optimum range of salinity. This is due to *Vibrio sp.* which are known as halophytic bacteria as they grow well in high-salinity aquatic environments, and their growth is inhibited when they are exposed to low-salinity water.

Liu and Chen (2004) showed that shrimp exposed to Ammonium stress prior to challenge showed higher susceptibility to *Vibrio sp.* According to the water quality data obtained in this study, alkaline pH conditions, high salinity and high ammonia and sulphide levels as well as low DO levels appear to create favourable conditions for the growth of *Vibrio sp.* in shrimp ponds (Heenatigala and Fernando, 2016). Similar results were observed in present study.

As vibriosis is a stress induced disease in shrimp (Jayasree, 2000) and most pathogenic *Vibrio sp.* are opportunistic pathogens, they become virulent in response to environmental effects that favour their survival over that of the host (Guzman et al., 2001). According to Palaniappan (1982), in any aquatic system, environmental parameters such as temperature, salinity, pH and dissolved oxygen play a foremost role in the distribution of bacteria. Sharmila et al., (1996) who stated that, pond as a confined environment is easy to be maintained by optimum physico-chemical parameters through proper pond management.

Conclusion:-

Based on present study, it is concluded that some physico-chemical parameters of shrimp pond may support the survival and growth of *Vibrio cholerae* and *Vibrio parahaemolyticus*. Pond conditions in shrimp farm may trigger the virulent potential of salt tolerant bacteria. *Vibrio cholerae* and *Vibrio parahaemolyticus sp.* are present in shrimp pond water, it is likely that they could also be present in the shrimp itself, with the implicit consumer health risk. At the same time, these physico-chemical parameters are required for successful shrimp growth. So, proper water management will reduce population of pathogens and provide a congenial pond environment for shrimp but prevention of bacterial infection would be difficult. However, results strongly suggested the need to conduct further studies regarding virulence factors, and other biotic and abiotic factors associated with *Vibrio sp.* for a better

understanding of their behaviour and commercially available antibacterial drug to control *Vibrio sp.* from pond for maintaining good pond environment.

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