ISSN (Print 0974-2115) (Online 2349-8552)
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Detection of WSS Virus on Banana Prawn, *Peneaus mergueinsis* By PCR Technique

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ABSTRACT

India, being one of the leading producers of shrimp's culture, *Peneaus mergueinsis* contributes about 61% of total world shrimp production. Shrimp growth and survival rates are strongly affected by the presence of several pathogens .White Spot Syndrome Virus (WSSV) greatly affects *P.merguinsis* and leads to high mortality. Prevention and inhibition of this virus in *P. merguinsis* have to be taken to raises our economic status. WSS affected Banana prawn is characterized by the presence of white spot in the exoskeleton and lead to the protein content losses have been estimated to the several million in different parts of India. Detection of this virus was done by PCR technique that is nested PCR which provides an increased sensitivity with conventional single primer pair PCR. This study shows that WSSV is found in Banana Prawn collected from south and north Nagappattinam & cuddalore. The percentage of infected Banana Prawn is mild 20% & carrier 6%. The main location of Nagappattinam and Cuddalore Islands are far away from mainland and brood stock is less susceptible to WSSV infection.

KEY WORDS: Banana prawn, WSSV, Nested PCR, Costal Area, Brood Stock.

1. INTRODUCTION

Aquaculture is the cultivation of the natural produce of water, such as fish or shellfish, algae and other aquatic organisms and also is a good source of foreign exchange. Banana prawn (*Penaeus merguiensis*) a euryhaline species distributed along the entire east cost of Africa, Taiwan, Madagascar, Pakistan, East and West coast of India, Srilanka, and Tamilnadu costal area. Banana prawn was affected by WSS virus that is white spot syndrome viruses which is characterized by the presence of white spots in the exoskeleton of infected shrimp. According to a recent world Bank report, global losses as a result of disease are around US $ 3000 million (Lundin, 1996) and losses have been estimated to be several million dollars in different parts of India (Anonymous, 1996). Prawns are most important exportable marine products in the global trade and also are a good source of foreign exchange India, being one of the leading producer of shrimp's culture, *P. merguinsis* contributed about 61% of total world shrimp production. Economic losses of *P. merguinsis* estimated to be US $ 3000 million *P.merguinsis* growth are affected by White Spot syndrome virus (wssv)

2. MATERIALS AND METHODS

Sample Collection: Two varieties of Prawn were collected form different landing centers in Tamil Nadu (Nagappattinam and Cuddalore).

Extraction of Shrimp DNA: DNA from the muscles tissue was isolated using SDS-phenol chloroform method as described by Lo (1996), with some modification The DNA was extracted with 1ml of extraction buffer (100 mM NaCl, 10mM Tris-HCl, pH 8.0, 50mM EDTA, pH 8.0, 05% Sodium dodecylsulfate and 0.1 Mg/ ml proteinase K).

Samples were incubated at 65^0C for 2 hours and centrifuged at 12000 rpm for 10 minutes. The supernatant were then extracted once with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) and twice with chloroform: isoamyl alcohol (24:1).

Identification of DNA by AGE was performed. DNA sample were loaded in to the slot of 0.8% agarose gel containing 0.5% Ethidium bromide. Extracted DNA were stored in TE buffer.

PCR Reaction: The Nested PCR kit from Genei developed by CIBA was used for PCR amplification. WSSV was detected by Nested PCR. Two steps were done 1st step PCR. 2nd step PCR.

1st STEP PCR: External primers were used.650 bp of viral genome were amplified.

2nd STEP PCR: Internal primers were used.350 bp of 1st PCR product were amplified. In 1st step PCR, 0.2µl Microfuge were used. To that, 23µl - 1st PCR premix, 1µl Taq polymerase,1µl Template DNA were added.S1-Positive control DNA,S2- Negative control DNA,S3- Control (Reagent mixture)These vials were kept at -20ºC for further amplification .1st PCR product were centrifuged for10000 rpm for 30 min to that 23 µl 2nd PCR premix was added**.** PCR products were analyzed by Electrophoresis. Protein Separation was carried out by SDS-PAGE.

Table.1. clinical Examination of WSSV in Banana prawn, *Penaeus merguiensis*

Location	Species	SL.No	M/F	Length(mm)	Weight (gm)	Clinical Sign of WSSV
Nagappattinam	*Penaeus merguiensis*	1	M	15	47.85	Two samples showed white spot on carapace out of 30 samples.
		2	M	16.5	30.15	
		3	M	19.5	42.41	
		4	M	21	49.30	
		5	M	18	71.53	
		6	F	18	37.55	
		7	F	21	55.45	
		8	F	17	72.55	
		9	F	21	69.70	
		10	F	20	86.55	
		11	F	22	72.50	
		12	M	19	73.56	
		13	M	20	69.25	
		14	M	20	85.62	
		15	F	19	66.59	
Average				19.33 ± 2.12	62.84 + 12.07	

3. RESULTS

Results shown that 12 crutacean's samples were positive for WSSV out of 30 samples examined (Table.3). The table.3, showed 4% positive in first step PCR where as second step 24% positive. Banana prawn, Penaeus merguiensis Nagappattinam shows 0% positive in first step PCR and 12% positive in second step PCR (Figures 1 and 2).

Figure.1. Banana prawn, *Peneaus mergueinsis* (Carrier of WSSV)

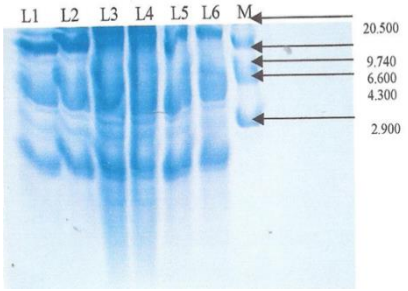

Figure.2. Molecular weight of muscle protein of *Peneaus merguensis*

Table.2. Clinical Examination of WSSV in Banana prawn, *Penaeus merguiensis*

Location	Species	SL.No	M/F	Length(mm)	Weight (gm)	Clinical Sign of WSSV
(Cuddalore)	*Penaeus merguiensis*	1	M	19	55.30	No sign of white spot on the carapace and other parts of the body.
		2	M	18	37.59	
		3	M	20	41.46	
		4	M	16	48.73	
		5	M	19	35.66	
		6	M	21	29.78	
		7	M	16	86.60	
		8	M	19.5	97.00	
		9	M	22	45.60	
		11	M	19	52.40	
		12	F	20	40.70	
			F	20.5	99.00	
Average				18.00±2.69	54.77±6.96	

Table.3. Detection of WSSV using PCR technique

Location	Type	Species	Total no. of	1st step	2nd step
(Nagappattinam)	Shrimp	Penaeis merguiesis	15	0	4
(Cuddalore)	Banana prawn	Penaeis merguiesis	15	+ve (2)	2
Total			30		12

Lane/band	Nagapattnam			Cuddalore			
	L1	L2	L3	L4	L5	L6	MW
1	8.069	16.751	18.398	18.398	18.398	20.078	20.500
2	6.458	7.632	8.069	8.069	8.522	9.978	9.740
3	3.534	3.292	3.245	3.245	3.230	7.632	6.600
4	1.423	1.423	1.347	1.347	1.347	3.984	4.300

4. CONCLUSION

Our study shows that the WSSV is found in Banana prawn, *Peneaus merguensis* collected from South and North Nagappattinam and Cuddalore. The percentage of positive in Banana prawn is mild (20%) and carrier (6%). It can also be observed that the presence of WSSV is low in Nagappattinam and Cuddalore, This may be the location of Nagappattinam and Cuddalore Islands which is far away from Mainland, and the brood stock is less susceptible to WSSV infection.

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