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Full Length Research Paper

Biosecuring of white spot syndrome virus on *Penaeus monodon* Fabricius, 1798

Sekar Ashokkumar, Packiam Mayavu* and Perumal Murugesan

Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai – 608 502, Tamil nadu, India.

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The present study was carried out with the aim of bio-securing of white spot syndrome virus (WSSV) on *Penaeus monodon* culture in grow-out and laboratory condition during the period of March to August 2010. In grow-out system, thirty six earthen ponds of varying sizes ranging from 0.5 to 1.0 ha were surveyed. Among 36 ponds, based on the differences in the management techniques, 15 were semi-intensive (group-I), 10 ponds were modified extensive (group-II) and 11 ponds as extensive (group- III). The results of the present study revealed that maximum average body weight (34.5 g), survival of 88% and proportion of pond success was 100% (15/15) with average culture period of 127 days in semi-intensive with probiotics applied ponds and the minimum average body weight (25.2g), survival of 79.1% and the proportion of pond failure was 27.2 (3/11) with average culture period of 70.7 days in extensive with regular management. In laboratory experiment, it showed that the cumulative mortalities occurred on 77th day in treatment-I and in control on 45th day and proportion of tanks failure was 100% (14/14) with average culture period of 70.7 days. Histopathological observation also revealed that hypertrophied nuclei was very low in 50 day of experiment than that of 77th day of experiment.

Key words: Histopathology, polymerase chain reaction, *Penaeus monodon,* probiotics, white spot syndrome virus.

INTRODUCTION

In the recent past, the shrimp farming has been facing serious problems and the production rate also decreased due to some viral diseases especially WSSV (White spot syndrome virus), SEMBV (systemic ectodermal mesodermal baculovirus) and MBV (monodon baculo virus) etc. White spot syndrome virus (WSSV) belongs to family Nimaviridae which contains a large circular double-stranded DNA genome of 292,967 bp (Van Hulten et al., 2001) but isolates with larger genomes have also been identified (AF440570) (Yang et al., 2001). WSSV virus are ellipsoid to bacilliform, enveloped particles with a distinctive tail-like appendage at one end and can be found throughout the body of the infected shrimp. They contain one nucleocapsid with a typical striated appearance and 5 major and at least 13 minor proteins (Huang et al., 2002; Van Hulten et al., 2000a, b, 2002). The wide spread and broad host range of WSSV continues to damage the shrimp culture industry worldwide. White spot syndrome in cultured shrimps is caused by white spot syndrome baculovirus, an enveloped non-occluded rod shaped virus (Flegal, 2006). This disease is characterized by the display of white spots on the exoskeleton (Chou et al., 1995; Lightner, 1996; Wang et al., 1995). Shrimps that are acutely affected by the WSSV show a rapid reduction in food consumption, lethargy, loose cuticle and pink to reddish-brown discoloration of hepatopancreas (Chou et al., 1995; Lightner, 1996). The white spots (0.2 to 0.5 mm dia.) are seen as abnormal calcium deposits on the exoskeleton resulting in mass moralities within 3 to 10 days. Although PCR (polymerase chain reaction) proved to be the most sensitive method to detect WSSV (Tapay et al., 1999; Yoganandhan et al., 2003), the lack of a set of universal primers and the absence of PCR products has made it essential to perform new viral purification

*Corresponding author. E-mail: Mayavu_p@yahoo.co.in. Tel: +91 9443098934. Fax: 04144-243089, 243070.

Figure 1. Map showing the study area.

and characterization with samples from WSSV outbreaks.

The histopathology of WSD (white spot disease) in moribund shrimps collected during outbreaks is distinctive and can be used for preliminary confirmation of an initial diagnosis. However, additional tests such as PCR, *in situ* DNA hybridisation, bioassay, transmission electron microscopy are required for final confirmation (OIE Manual of Aquatic, 2009). The herbal plants such as *Calotropis procera*, *Psoralesa corylifolia*, *Pongamia glabra* and *Psidium guajava* were extracts having antiviral and immunostimulantes help to enhance the resistance against WSSV infection (Kumaran et al., 2009). Biosecure system could be based on specific pathogen-free stocks including enclosed, reduced water-exchange/increased water-reuse culture systems, biosecure management practices and co-operative industry-wide disease control strategies. The aforementioned trend of aquaculture, the present study was made to find out the production and growth rate of WSSV-infected and WSSV-free *P. monodon* reared in the grow-out ponds and laboratory.

MATERIALS AND METHODS

The present study was carried out in Nandaset and Vallivilas aqua farms located on the southern bank of Vellar estuary (Latitude 11° 29' N, Longitude 79° 46' E), Parangipettai, Tamilnadu, India (Figure 1).

Totally, thirty-six earthen ponds of varying size ranging from 0.5 to 1.0 ha were surveyed. Among 36 ponds, based on the difference in the management techniques, 15 were considered as semi-intensive (group-I), 10 ponds as modified extensive (group-II) and 11 ponds as extensive (group-III). The 14 tanks (laboratory) were considered as group- IV. Observations were made during the entire period of culture lasting for 150 days falling from March to August 2010.

Group-I

The ponds were stocked with WSSV- negative PL (post larvae) 15 to 18 (10 to 15 ind. m^{-2}) purchased from a commercial hatchery (Sona shrimp marine harvest, Marakkanam). Water exchange was carried out using the water from a relatively virus-free pretreated reservoir and bore well. Fences were kept using the nylon nets (mesh size 10 mm) around the pond to avoid entry of crab into the ponds. Probiotics were applied from 15 to 60 days of culture once in a week.

Activation of probiotics

Commercially available probiotics-Super NB (microbial culture) (350 ml) was mixed with 60 to 65 L of pond water and aerated continuously for 4 h using battery aerator. After activation, the slurry was applied evenly throughout the pond water. During application, the paddle wheel aerators were operated for thorough mixing.

Application of chemicals

Commercially available Semvac-P (10 g/kg of feed) and vitamin-C (5 g/kg of feed) were mixed with pellet feed and broadcasted evenly at 2nd and 4th feeding schedule respectively. Superbiotic (10 g/kg of feed) and Nutrigel (20 ml/kg of feed) were also mixed with pellet feed, shade-dried and given in the 2nd feeding schedule. Aquolact (5 g/kg of feed) and Soin and Stroner (5 g/kg of feed) were mixed with pellet feed and broadcasted in the 2nd and 5th feeding schedule (Table 1).

Group-II and III

Ten ponds of modified extensive culture and 11 extensive ponds were used in groups-II and III respectively. The ponds were stocked with WSSV-negative PL 15 to 18 at a density of 6 to 10 pieces m^{-2} and 4 to 5 pieces m^{-2} respectively in groups II and III. Water was drawn from vellar estuary.

Table 1. Application schedule of chemicals for group-I.

DOC	Chemical	Concentration (g/kg)	Time of application
31 to 125	Superbiotic	10	1st feeding
31 to 125	Vitamin-C	5	2nd feeding
50 to 100	Aquolact	5	2nd feeding
70 to 60	Semvac-P	10	4th feeding
80 to harvest	Soin and Stroner	5	5th feeding

Table 2. Application schedule of chemicals for groups-II and III.

DOC	Chemical	Concentration (g/kg)	Time of application
30 to 120	Growel	5	1st feeding
30 to 120	Immunol	5	4th feeding
40 to 80	Sugar	2	2nd feeding
30 to harvest	Vitamin-C	5	2nd feeding

Application of chemicals

Commercially available growel (5 g/kg of feed) and immunol (5 g/kg of feed) were mixed with pellet feed and shade-dried. The mixed feed was broadcasted at 1st and 4th feeding schedule. Sugar (100 g/kg of feed) and vitamin-C (5 g/kg of feed) were mixed with pellet feed and shade-dried. After complete drying, the feed was broadcasted evenly during 2nd feeding schedule (Table 2).

Laboratory experiment (group-IV)

Post larvae (PL) -15 were purchased from commercial hatchery (Sona shrimp marine harvest, Marakkanam) and screened for WSSV using 2 step PCR test (Chang et al., 1996). The WSSV-positive post larvae (PL) were brought to the laboratory and stocked in 50 L capacity culture tanks having 35 L chlorinated filtered estuarine water. Fifty positive PL-15 were stocked in the control, 150 each in treatments-I and II. The animals were provided with aeration and feeding was given with 7.5% of body weight; initially, subsequently feeding was adjusted to 5 to 3.5% of body weight per day considering the left-out, unfed feed and increased body weight of animals. The feed was given four times a day (60% at 6.00 am and 12 noon and 40% at 6.00 pm and 10 pm). In the experimental tanks, salinity was measured by using hand refracto-meter (Erma, Japan); pH by using a calibrated pH pen (pH ep-3 model); temperature by a mercury thermometer having ± 0.0°C accuracy; and dissolved oxygen by modified winkler's method as described by Strickland and Parsons (1972). The bottom water in the tanks along with excess feed and faeces was siphoned out using 2 cm through plastic hose daily.

The behavior of animals was also observed everyday and ten animals were collected randomly every week from each tank for measuring total wet weight.

Polymerase chain reaction (PCR)

WSSV infection PL was confirmed by PCR technique. Template DNA was extracted following the procedures of Vijayan et al. (1998). PCR products were electrophoresed in 0.8% agarose gel stained with ethidium bromide and visualized under ultraviolet transillumination.

Histology

The experimentally dead animals were dissected immediately and the gut was separated. These were fixed in Davidson's fixative for 24 h and then transferred to 90% alcohol and processed using routine histological techniques (Bell and Lighter, 1988). Paraffin sections of 4 to 5 μm thickness was stained with Hematoxylin and Eosin and examined microscopically. Photomicrographs were taken using a wild Mps 46 micro camera fitted with Leitz laborlaxs microscope.

Crude extraction of plants and animals material

Leaf samples collected from neem and phyllanthus plants were extracted for methanol in 48 h by following cold percolation method and evaporated under room temperature of 26 ± 2°C. The resultant brown residue was dried. The dried sample was then powdered using a pestle and mortar (Table 3).

Application of chemicals of plant extract

The plant extract powder (2 to 5/100 g of feed) was mixed with commercial pellet feed and shade-dried given in the 1st feeding schedule. Liv-52 and Albadot were mixed separately with fish oil and coated over the feed at the rate of 2 g/kg of feed and shade-dried given in the 3rd feeding schedule.

RESULTS

Group-I

The results revealed that moderate (240%) water exchanges, maximum average body weight of 34.5 g (Figure 2), the maximum average survival of 88% (Figure 3) and 15 ponds were terminated in the last 4th and 5th months of culture. The proportion of pond success of100% (15/15) and average culture period was 127 days (Figure 4). Two-way ANOVA showed significant differences

Table 3. Application schedule of plant powder extract and antibiotics.

DOC	Treatment-1		Treatment-2		Schedule of feeding
	Neem extract and Liv 52	Conc./100 g of feed	Phyllanthus extract and Albadot	Conc./ 100 g of feed	
1-7	-	-	-	-	-
7-15	Neem extract powder (2 times)	3 g	Phyllanthus extract powder (2 times)	3 g	1st
15-22	Liv 52	150 mg	Phyllanthus extract (2 times)	3 g	1st
22-30	Liv 52 (2 times)	150 mg	Albadot (3 times)	400 mg	3rd
30-38	Neem extract powder (2 times)	50 mg	Phyllanthus extract (once)	5 g	1st
38-45	Neem extract powder (2 times)	5 mg	Phyllanthus extract (3 times)	5 g	1st
45-53	Liv 52 (2 times)	200 mg	Phyllanthus extract (3 times)	6 g	1st
53-60	Neem extract powder (2 times)	5 g	Albadot (2 times)	500 mg	3rd
60-67	Liv 52 (2 times)	200 mg	Phyllanthus extract (once)	6 g	1st
67-77	Neem extract powder (2 times)	5 g	-	-	1st

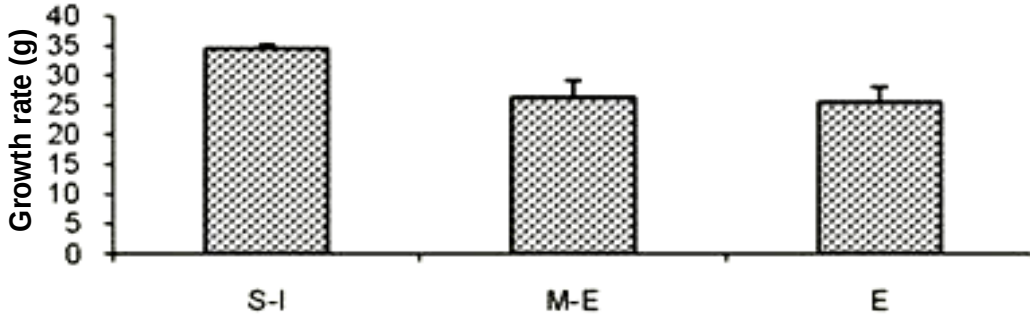

Figure 2. Growth rate for different methods of culture. Note: SI- semi intensive, ME- modified extensive and E- extensive.

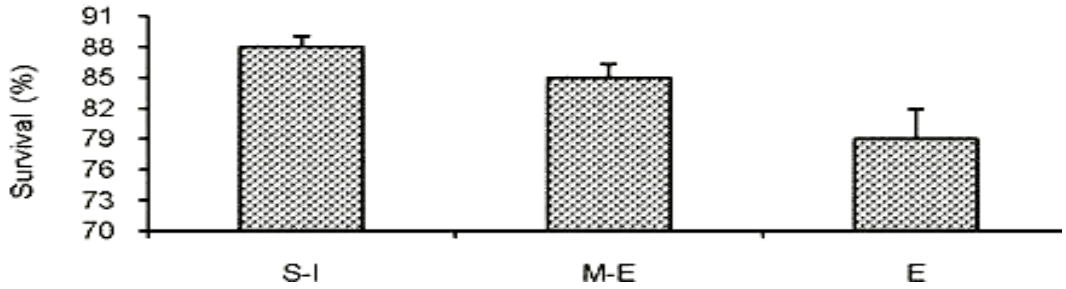

Figure 3. Survival rate for different methods of culture.

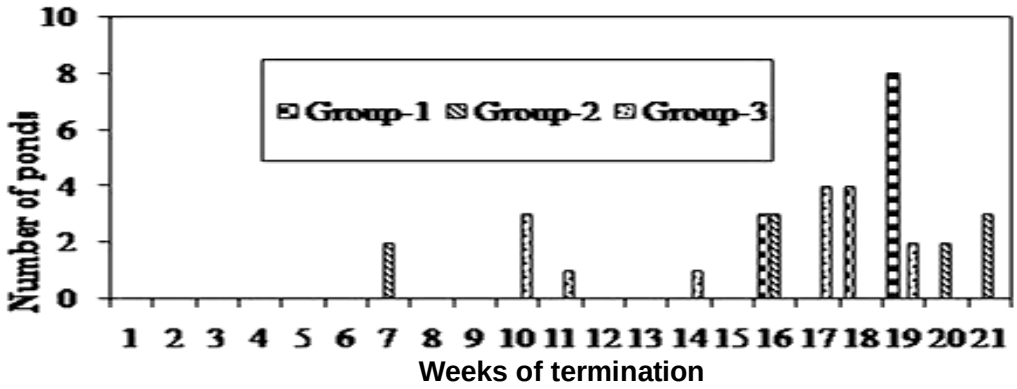

Figure 4. Group 1, 2 and 3 WSSV - negative PL.

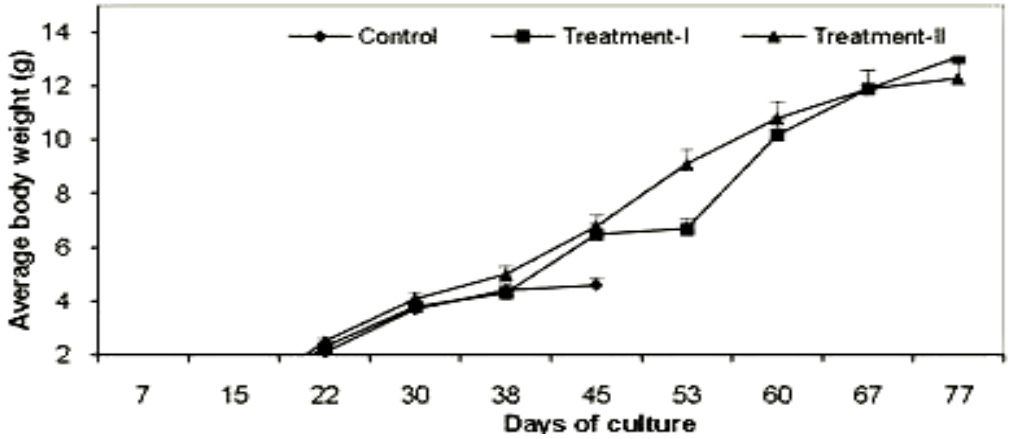

Figure 5. Growth rate for different treatments.

at 0.05% level of growth and survival between the days of culture and types of culture.

Group-II

The maximum (390%) water exchange, maximum average body weight of 26. 2 g (Figure 2) and maximum average survival of 88% (Figure 3). Of all the 10 ponds, four was failure while 6 were successful and the proportion of the failure rate was 40% and average culture period for failure and successful ponds was 94.5 and 134 days respectively (Figure 4). Two-way ANOVA showed significant differences at 0.05% level of growth and survival between the days of culture and types of culture.

Group-III

The maximum (170%) water exchange, maximum average body weight of 25. 2 g (Figure 2) and maximum average survival of 79.2% (Figure 3). Of all the 11 ponds, three were failure while 8 were successful and the proportion of the failure rate was 27.2% and average culture period for failure and successful ponds was 67 and 105 days respectively (Figure 4). Two-way ANOVA showed significant differences at 0.05% level of growth and survival between the days of culture and types of culture.

Laboratory experiment (group-IV)

Physio-chemical parameters were maintained with optimum level during the study period. The temperature ranged from 30 to 32°C, salinity 28 and 30 psu, pH 7.8 to 8.0 and dissolved oxygen 4.2 and 4.6 mg/l and cumulative mortalities occurred in treatment-I on 77th day of experiment followed by 73rd day in treatment-II and 45th day in control. The maximum growth of 13.1 g (77 days) was recorded in treatment-I and minimum of 4.6 g (45 days) in control (Figure 5). Similarly, maximum survival of54% was observed in treatment- I followed by in treatment- II with 52 and 48% in control (Figure 6). All the

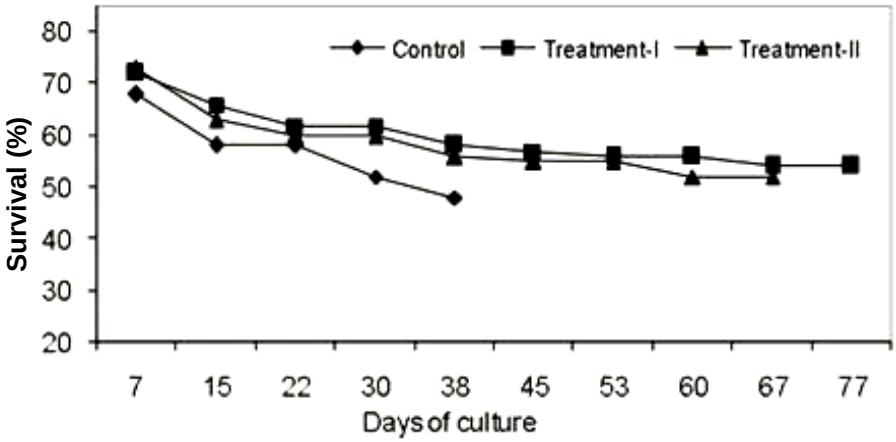

Figure 6. Survival rate for different treatments.

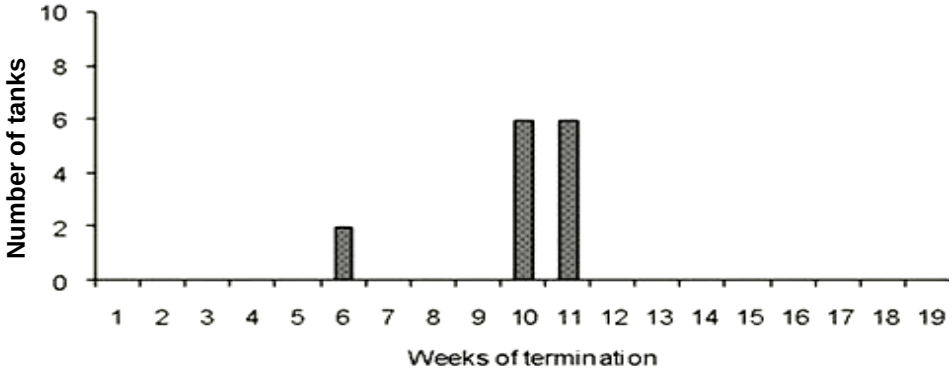

Figure 7. Experiment II WSSV-positive PL.

tanks were terminated in second and third month of experiment and proportion of failure was 100% (14/14). The average culture period was 75 days in treatment and control 44 days (Figure 7). Histopathology showed the very low hypertrophied nuclei of gut during 30 days in experimental infected animals (Plate 1a) and compared to after disease outbreak (Plate 1b). In the early stage of infection, animals were observed cannibalizing dead infected shrimps, promoting the spread of WSSV (Plate 2).

2-step PCR test

The 2-step PCR test revealed that lanes-1 and 3 was WSSV-positive and negative control marker respectively and lane-2 was whole body of shrimp post larvae (Plate 1c). The result of the present work showed the bands of lane-2 formed equal to standard WSSV positive control marker.

DISCUSSION

Generally, pond bottom water is drained to remove the organic load and top water is removed to avoid the excess bloom. The rate of water exchange is determined by shrimp biomass, feed intake, phytoplankton density and microbial load. In the present study, maximum (390%) water exchange was done in the modified extensive ponds. Chen et al. (1988) suggested that daily water exchange of 30% is in a super-intensive culture system. Among the ponds, the maximum average body weight of 34.5 g, survival of 88% and the proportion of pond success of 100% (15/15) was observed in probiotics applied semi-intensive ponds. Koshio (1985) reported that higher growth rate was related to higher moult frequency. Reasons for 100% success might be due to the application of probiotics and also good farm management such as the use of borewell water and crab protection fences. Generally, shrimps have weak immune system than fishes and are relatively more dependent on the non-specific immune process such as phagocytosis, which plays an important role in non-specific defense mechanism against pathogen. In the present study, the application of probiotics in water has improved the immune potentiating capacity by increasing phagocytosis in shrimps. This view is in support of Pollmann et al. (1980) and Raa et al.

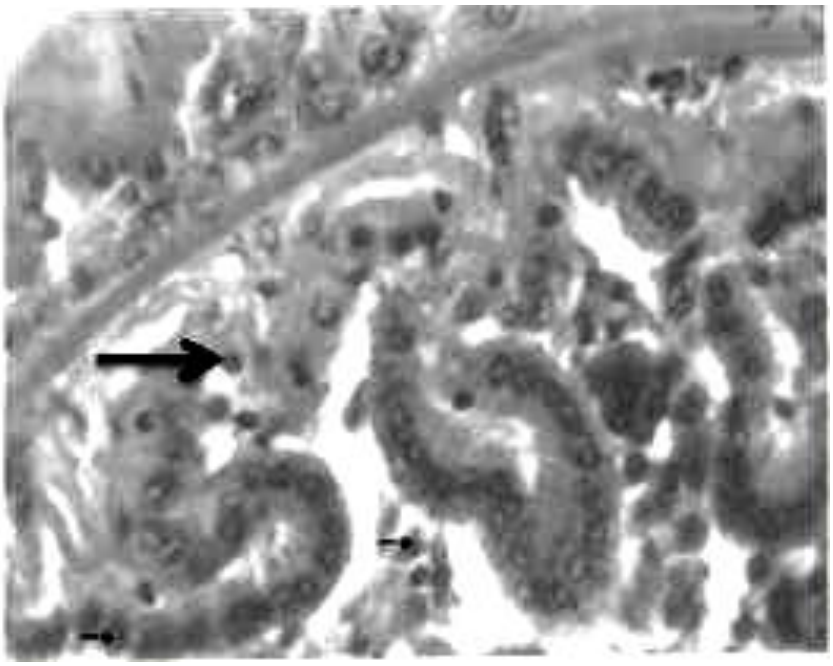

Plate 1a. Hypertrophic nuclei marked in (arrow) in 50 days of WSSV infected animal (200 x).

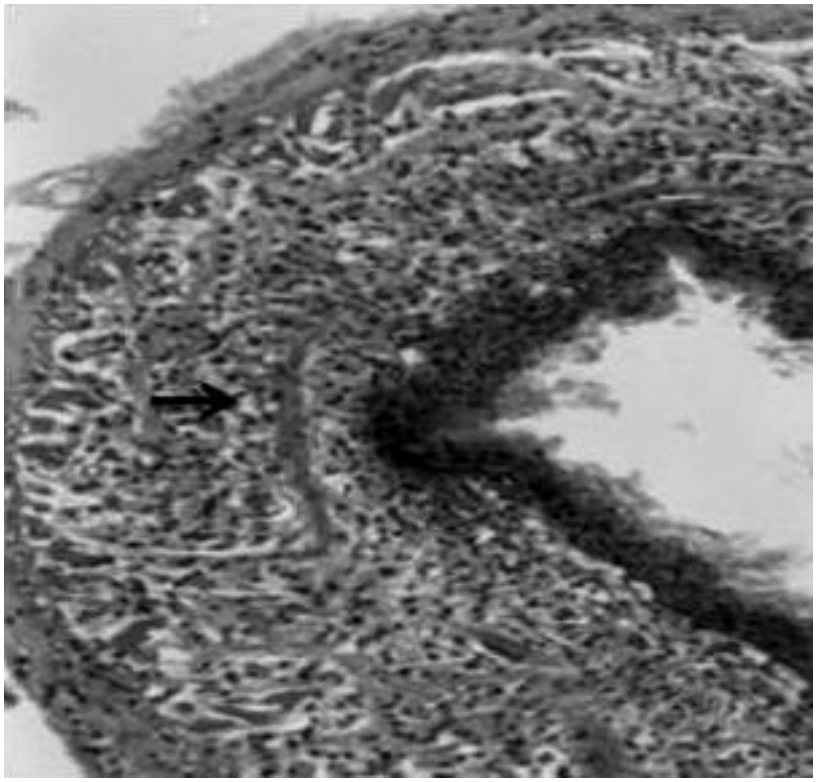

Plate 1b. Hypertrophic nuclei marked in (arrow) after disease outbreak (200 x).

(1992). The probiotic organisms produce specific components like bacteriocins which are known to inhibit pathogens including *Straphylococcus aureus, Aeromonas hydrophila* and *Listeria monocytogens* (Lewus et al., 1991).

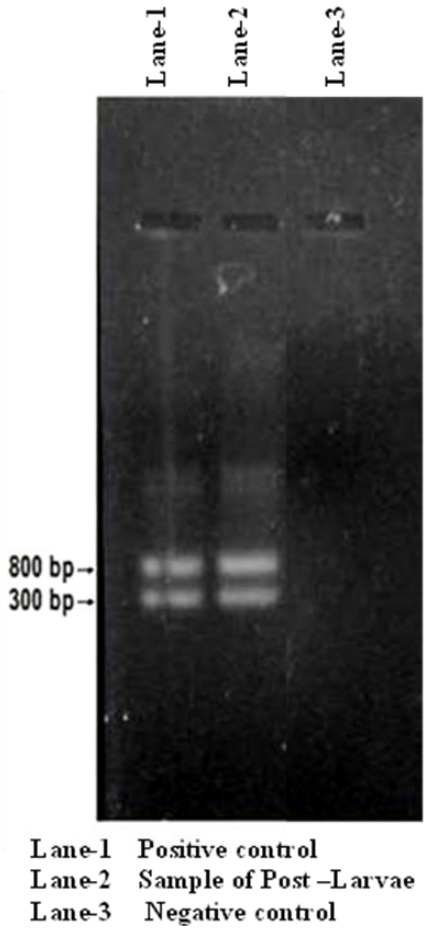

Plate 1c. 2- step PCR test of shrimp larvae.

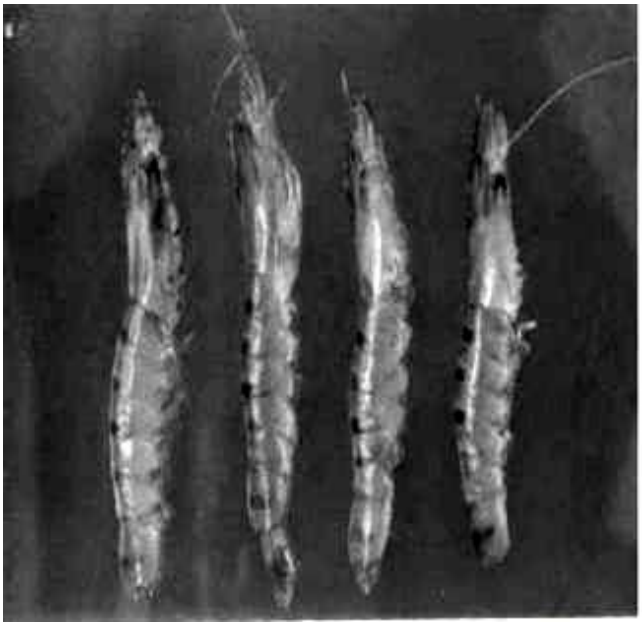

Plate 2. Infected *P. monodon* showing loss of parts due to cannibalism.

Though, the pathogens cannot be eliminated in total from the culture system, the beneficial bacteria established through the probiotics may suppress the growth of the same (Maeda and Nogmi, 1989). In groups-II and III, ponds stocked with WSSV-negative PL, the proportion of failure was 40 and 27.2% respectively. This may be due to false negative PL screening due to either low sensitivity of the PCR technique used or the sampling error. The error could be reduced by increasing the sampling frequency or the sample number. Another reason is that WSSV might have been horizontally transferred namely neighboring infected ponds or by unknown carriers. The pattern of disease spread suggested that the virus could be transferred amongst ponds by water-borne vectors, water seepage or poor sanitary practices (for example use of shared nets, boats or other equipments) spread by crabs moving among ponds as reported by Subamattaya et al. (1988). Also, groups-II and III ponds were not provided with crab protection fences. WSSV disease outbreak was observed mostly during the 3rd month of cultivation of negative PL stocked ponds. The reason is that most of the pond operators do not exchange water for the first 30 day of culture and this should drastically reduce the risk of horizontal transfer of the virus up to the second month (Withyachumnarnkul, 1999). The outbreak during first 40 to 45 days of cultivation probably occurred through stocked PL and not through horizontal transmission. This was further proved by the present laboratory culture stocked with WSSV-positive PL developed into WSSV outbreak 43 to 45 days in control.

The behavior of experimental cultured animals showed original pigmentation, intense feeding, normal faecal production and no white spots on the carapace and appendages but after infection, there was intense red pigmentation in the whole body, with characteristic white spots on the exoskeleton. The infected shrimps became lethargic with reduced feeding activity, broken and partially digested faecal strands. The infection intensified with the events that the shrimp stopped feeding, surfaced frequently and exhibited loss of balance and cumulative mortality within 2 to 3 days. Similar mass mortality caused by WSBV was reported in the cultured shrimps from Taiwan (Chou et al., 1995) and in India (Rajan et al., 2000). In the present study, early stage of infection, some animals were observed cannibalizing dead infected shrimps and promoting the spread of WSSV in the population. Histopathology result revealed that hypertrophied nuclei was present in gut of experimental animal. Chang et al. (1996) also reported nuclear hypertrophy, cell lysis and tissue degeneration in the experimentally infected *P. monodon.* The present study supports the application of plant extract and antiviral compounds like Albadot in aquaculture ponds because the experimental results showed that the mortality was extended minimum of 55 to 60 days compared to control. Neem extract powder has been used against white spot

disease of shrimp (Srinivasan and Bragadeeshwaran, 2001). The modes of action of Albadot are not allowing the viron to settle on the host, inhibiting the elongation of capsid, stopping cell lysis, inhibiting the genetical replication and depolarization and inhibition of cell wall formation etc. The present formulation is the first of its kind which has prophylactic and therapeutic properties against WSSV in penaeid shrimps. However, this trail was carried out under *in situ* condition and therefore, field trials have to be conducted to work out the effective dosage and frequency of application depending up on the type of culture practice, density of stocking, and intensity of infection etc.

Moreover high stocking density with probiotics and low stocking density perhaps led to the successful harvest from culture period extending more than 100 days. With regard to management of diseases like white spot syndrome, a multi-prolonged management strategy would be very essential. Brood stocks are to harbour the virus in copepods, crabs, pest prawns etc., occurring in the environment (Otta and Karunasagar 1999). White spot syndrome virus has a very broad host range in decapods crustaceans. In marine shrimp, the virus occurs commonly as a low-level persistent infection in the absence of clinical signs, but rapid by increases in viral load, precipitated by physiological stress, salinity change or lower temperatures can lead to disease and mass mortalities in ponds (Peng et al., 1998; Aranguren et al., 2001; Du et al*.,* 2006; Granja et al., 2006; Liu et al., 2006). There is ample evidence that WSSV replicates most efficiently at 23 to 28°C (Guan et al., 2003; Du et al., 2006; Granja et al., 2006; Reyes et al., 2007) and high water temperatures prevent the onset of disease (Rahman et al., 2006). The virus also survives for longer periods in seawater at lower temperature (Momoyama et al., 1998) and seawater temperature <30°C are conducive to higher prevalence of WSSV infection in wild shrimp populations (Rodriguez et al., 2003; Withyachumnarnkul et al., 2003). It is reported that other invertebrates such as polychaetes, bivalves, rotifers, artemia, copepods and some insect larvae as well as microalgae can also accumulate high levels of viable WSSV in the absence of demonstrated virus replication and may act as mechanical vectors of infection (Yan et al., 2004; Liu et al., 2007). Recent research on zooplankton has shown that may be a vector for the transmission of WSSV (Mang et al., 2007; Zhang et al., 2008).

Avoidance of the virus is almost impossible. But it is better to improve the immune status of the host by using immunostimulants by keeping the good water quality by using proper feed management, water exchange and bio-remediators or probiotics. Proper eco-friendly strategies on disease management are possible ways to revive shrimp culture industry and also screening of PL for WSSV status before stocking. The proportion of successful harvest for WSSV-negative PL batches in the present programme described was approximately 80.05%. The present study concludes that proper management in shrimp culture ponds is necessary for achieving a good and successful production and survival and the experimental results showed that the mortality was extended minimum 55 to 60 days compared to control. Therefore, the herbal plants neem and phyllanthus having anti viral and immunostimulant activity help to enhance the resistance against WSSV infection and they can be recommended for shrimp culture.

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REFERENCES

Aranguren F, Brock JA, Granja CB, Salazar M, Vidal OM (2001). A profound effect of hyperthermia on survival of *Litopenaeus vannamei juveniles* infected with white spot syndrome virus. J.W. Aquaculture Society. 32: 364 – 372.

Bell TA, Lightner DV (1988). A handbook of normal *penaeid* shrimp histology. World Aquaculture Society, Aquaculture development program state of Hawaii. Allen Press, Lawrence, p. 114.

Chang PS, Kou, GH, Lo, CF, YC Wang (1996). Identification of white spot syndrome associated baculovirus (WSBV) target organs in the shrimp *P. monodon* by in-situ hybridization. Dis. Aquat. Org., 27: 131 - 139.

Chou HY, Chiang HC, Huang CY, Lo CF, Wang CH (1995). Pathogenecity of baculovirus infection causing white spot syndrome in cultured *penaeid* shrimp in Taiwan. Dis. Aquat. Org., 23: 165 - 173.

Du HH, Li WF, Ki, ZS, Xu ZR (2006). Effect of hyperthermia on the replication of white spot syndrome virus (WSSV) in *Procambarus clarkia*. Dis. Aquat. Org., 71: 175 – 178.

Flegal TW (2006). The special danger of viral pathogens in shrimp translocated for aquaculture. Science Asia, 32: 215 – 231.

Granja CB, Parra G, Salazar M, Vidal OM (2006). Hyperthermia reduces viral load of white spot syndrome virus in *Penaeus vannamei*. Disease aquatic Organfisms. 68: 175–180.

Guan Y, Li C, Yu Z (2003). The effects of temperature on white spot syndrome infections in Marsupenaeus *japonicus*. J. Inver. Pathol., 83: 257 – 260.

Huang C, Hu ZH, Hew C L, Zhang X, Lin Q, Xu X (2002). Proteomic analysis of shrimp white spot syndrome viral proteins and characterization of a novel envelope protein VP466. Mol. Cell Prot., 1: 223 – 231.

Koshio S (1985). The effect of eyestalk ablation, and environmental factors on growth, survival and energy utilization of juvenile American lobsters, *Homarus americanus* as applied to aquaculture. Ph.D. Thesis. Dalhous University, Halifox, Canada. p. 218.

Kumaran T, Venkatramalingam K, Punitha SMJ, Babu MM, Velmurugan S, Thangaviji V, Citarasu T (2009). Conference on recent advanced in applied Zoology. P A18: 25.

Lewus CB, Kaiser A, Montville JJ (1991). Inhibition of food borne bacterial pathogens by Bacteriocins form Lactic Acid Bacteria isolated from Meat. Appl. Microbiol., 57: 1683 -1688.

Lightner DV (1996). A handbook of shrimp pathology and diagnosis procedures for diseases of cultured *penaeid* shrimp. World Aquaculture Society, Baton Rouge.

Liu B, Guan Y, Song X, Yu Z (2007). Studieson the transmission of WSSV (white spot syndrome virus) in juvenile *Marsupenaeus japonicus* via marine microalgae. J. Invert. Pathol., 95: 87–92.

Liu B, GuanY, He J, Jian X, Song X, Yu Z (2006). The effect of acute salinity change on white spot syndrome (WSS) out-breaks in *Feneropenaeus chinensis*. Aquaculture. 253: 163 – 170.

Maeda M, Nogmi K (1989). Some aspects of the biocontrolling methods in aquaculture. *In* Miyachi S, Karoke I, Ishida Y (eds.) current topics in marine biotechnology, Japan Soc. Mar. Biotechnol. Tokyo. 437.

Mang JS, Dong SL, Dong YW, Liu XY, Tian XL, Yan DC (2007). *Virus*-Oceanologica Sinica. 26: 109 - 115.
Momoyama K, Hiroka M, Nakano H, Sameshima M (1998). Cryopreservation of penaeid rod shaped DNA virus (PRDV) and its survival in sea water at different temperatures. Fish Pathol., 33: 95 – 96.
Otta SK, Karunasagar I (1999). Bacterial flora associated with shrimp culture ponds growing *P. monodon* in India J. Aquat. Tropics. 14(4): 309 - 318.
Peng SE, Lo CF, Liu KF, Kou GH (1998). The transition from pre-patent to patent infection of white spot syndrome virus (WSSV) in *Penaeus monodon* triggered by periopod excision. Fish. Pathol., 33: 395 – 400.
Pollmann DS, Danielson DM, Pbo ER (1980). Effects of microbial feed additives on performance of starter and growing finishing pigs. J. Anim. Sci., 51: 577 - 581.
Raa J, Roerstad G, Engstad R, Robertson B (1992). The use of immunostimulants to increase resistance of aquatic organisms to microbial infections. In: Sahriff, M., Subasinghe, R.P., Arthur, J.R. (Eds)., Diseases in Asian Aquaculture 1. Fish Health Section, Asian Fisheries Society, Manila, Philippines, pp. 39–50.
Rajan PR, Bernnan GP, Ramasamy P, Purushothaman V (2000). White spot baculovirus syndrome in the Indian shrimp *P. monodon* and *P. indicus*. Aquaculture. 184: 31 - 44.
Reyes A, Granja C, Salazar M (2007). Temperature modifies gene expression in subcuticular epithelial cells of white spot syndrome virus-infected *Litopenaeus vannamei*. Dev. Comp. Immunol., 31: 23 - 29.
Srinivasan M, Bragadeeswaran S, Rajaram R (2001). Role of Neem Cake, Neem powder in Aquaculture. *Vol. IV*.
Strickland JDH, Parsons TR (1972). A practical hand book of sea water analysis. Bull. Fish Res. Bd. Canada, 167: 311.
Tapay LM, Loh PC, Nadala Jr, EC (1999). A polymerase chain reaction protocol for the detection of various geographical isolates of white spot virus. J. Virol., 81: 307 – 316.
Van Hulten MCW, Goodall SD, Vlak JM, Westenberg M (2000b). Identification of two major virion protein genes of white spot syndrome virus of shrimp. Virology, 266: 227 – 236.
Van Hulten MC, Fiers WM, Kloosterboer N, Klein Lankhorst R, Witteveldt J, Peters S, Sandbrink R, Tarchini H, Vlak JM (2001). The white spot syndrome virus DNA genome sequence. Virology, 286: 7 – 22.
Van Hulten MCW, Goodall SD, Vlak JM (2000a). Three functionally diverged major structural proteins of white spot syndrome virus evolved by gene duplication. J. Gen. Virol., 81: 2525 – 2529.
Van Hulten MCW, Reijns M, Vermeesch AMG, Zandbergen F, Vlak JM (2002). Identification of VP19 and VP15 of white spot syndrome virus (WSSV) and glycosylation of the WSSV major structural proteins. J. Gen. Virol., 83: 257 – 265.
Vijayan KK, Rajendran KV and Santiago TC (1998). A simple and rapid molecular diagnostic technique for white spot syndrome virus (WSSV) infection. In: Balachandran K.K. (ed.), Advances and Priorities in Fisheries Technology. Society of Fisheries Technologists (India), Cochin, pp. 398–401.
Wang CH, Lo CF, Leu JH, Chou CM (1995). Purification and genomic analysis of baculovirus associated with white spot syndrome WSBV of *Penaeus monodon*. Dis. Aquat. Org., 23: 239 – 242.
Withyachumnarnkul B (1999). Result from black tiger shrimp, *P. monodon* culture ponds stocked with postlarvae, PCR-positive or negative for white-spot syndrome virus (WSSV). Dis. Aquat. Org., 39: 21 - 27.
Withyachumnarnkul B, Boonsaeng V, Chomsoong R, Flegel TW, Muangsin S and Nash GL (2003). Seasonal variation in white spot syndrome virus-positive samples in broodstock and post-larvae of *Penaeus monodon* in Thailand. Dis Aquat. Org., 53: 167 - 171.
Yan DC, Dong SL, Feng MY, Huang J, Liu XY, Yu XM (2004). White spot syndrome virus (WSSV) detected by PCR in rotifers and rotifer resting eggs from shrimp ponds. Dis. Aquat. Org., 59: 69 - 73.
Yang F, He J, Lin X, Li Q, Pan D, Xu X, Zhang X (2001). Complete genome sequence of the shrimp white spot bacilliform virus. J. Virol., 75: 11811 – 11820.
Yoganandhan K, Murugan RB, Narayanan AS, Sathish S, Sahul Hameed AS (2003). Screening the organs for early detection of white spot syndrome virus in *Penaeus indicus* by histopathology and PCR techniques. Aquaculture. 215: 24 – 29.
Zhang JS, Dong SL, Dong YW, Hou CQ, Tian XL (2008). Bioassay evidence for the transmission of WSSV by the harpacticoid copepod *Nitocra sp.* J. Invet. Pathol., 97: 33 - 39.