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# First report on White Spot Syndrome Virus (WSSV) infection in white leg shrimp *Litopenaeus vannamei* (Crustacea, Penaeidae) under semi intensive culture condition in India

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**Abstract**. Scientific shrimp culture began in India in the late eighties along the east coast particularly in Andrapradesh and Tamilnadu. Continuous success of shrimp culture was affected by mass mortalities of cultured shrimp in 1994. Thereafter disease infection on survival and production of shrimps get its importance in culture. The present study is the first report on WSSV (white spot syndrome virus) infection in cultured *Litopenaeus vannamei* (Boone, 1931) in India. WSSV infection was observed on 70$^{th}$ days of culture due to cross contamination of white spot infected shrimp from the neighboring farm because of birds. Due to this infection within two days the mortality ratio has gone up to 25% in pond 1 and 12% in pond 2. So this present study strongly recommends to every shrimp farmers to go for bird fencing & crab fencing to avoid horizontal contamination, before stocking the good quality seed, then they will have the risk free WSSV culture.
**Key words**: *L. vannamei*, WSSV, India, biosecure methods, bird fencing, crab fencing.

**Introduction**. Shrimp farming is a multi-billion dollar industry contributing a major income to several countries in Asia and South America. The rapid growth of shrimp farming led to an economic boom but, unfortunately, the outbreak of viral diseases has increased the economic risks and slowed the industry development (Flegel 2006). White spot disease (WSD), commonly known as the white spot syndrome virus (WSSV) is an important viral pathogen and it is responsible for huge economic losses in the shrimp culture industry worldwide (Lightner 1996; Flegel 1997). The WSSV is an enveloped, double stranded DNA virus, ovoid to bacilliform in shape with a tail like extension at one end (Van Hulten et al 2001; Yang et al 2001). The virus is the only one member of the family Nimaviridae, genus *Whispovirus* (Mayo 2002).

The first outbreak due to WSSV was reported in shrimp farms in Taiwan in 1992 (Chou et al 1995) followed by other shrimp farming countries of South East Asia, Middle East, North, Central and South America (Lightner 1996; Rodriguez et al 2003; Flegel 2006). In India, WSSV was first reported in 1994 on black tiger shrimp (*Penaeus monodon* Fabricius, 1798) from Visakhapatnam of Andhra Pradesh to Sirkali of Tamilnadu covering a very large area (Anonymous 1994; Shankar & Mohan 1998). Currently, the most serious shrimp viral pathogen in the world is WSSV; it is a sole pathogen and it may cause disease and mass mortality 100 % within 2-10 days after the onset of symptoms (Lightner 1996; Xu et al 2006). At present, no treatments are available to cure the disease and mortality. The only alternative to reduce the risk of WSSV is the implementation of bio-security or exclusion measures, such as filtration and disinfection

(Clifford 1999; Lightner 2005). Water is a major probable pathway for WSSV entry into an aquaculture facility (Lotz & Lightner 1999; Cohen et al 2001; Corsin et al 2005). Currently in shrimp farming, pond wastewater is routinely discharged into the adjacent environment (coastal lagoons or estuaries), where other crustacean species dwell. Many crustaceans are potentially susceptible to WSSV infection (Escobedo-Bonilla et al 2005). Heavy water exchange is normally done even in the ponds affected by WSSV outbreaks. These practices probably increase the risk of WSSV transmission to neighboring shrimp farms.

The first clinical sign of WSSV is the presence of white spots on the carapace. The body colour of diseased shrimp becomes pale or reddish in colour (Takahashi et al 1994). Exposure of shrimp to stress increases the risk of WSSV, since stressors compromise the shrimp immune system (Takahashi et al 1995). Consequently, under stressful conditions, WSSV can proliferate rapidly and cause mortality (Lo & Kou 1998; Doan et al 2009). WSSV outbreaks may be preceded by or coincide with high pH and un-ionized ammonia in shrimp pond water (Corsin et al 2001). Fluctuations in salinity and temperature can weaken the shrimp's immune system and enhance the viral replication. Variation in water temperature increases the WSSV infection and the outbreak of clinical disease. Mortality was 100%, however, in WSSV-infected shrimp transferred from 32°C to 25.8±0.7°C (Vidal et al 2001). In India, so far there is no report regarding WSSV infection in *Penaeus vannamei* (Boone, 1931). The present research aimed to study the culture of the white leg shrimp (*Litopenaeus vannamei* (Boone, 1931)) without water exchange and the importance of biosecure methods to avoid horizontal contamination against WSSV. This study is the first report in India about the *L. vannamei* cultured shrimps infected by WSSV.

**Material and Methods**. This study was performed at a shrimp farm located in Bhimavaram, west Godavari district, Andhrapradesh, India. The study was conducted in two shrimp culture ponds (pond no 1 and 2). The size of the pond no 1 was 0.96ha and 1.2m deep and pond no 2 was 2.4 ha and 1.5m deep. The soil type of the ponds was sandy clay in nature. Besides the above ponds culture system had a reservoir pond in the size of 2ha, a sedimentation and chlorination ponds were in the size of 0.6 ha each. Pumps are used for water recirculation from the culture ponds to sedimentation pond. The water in the sedimentation pond was allowed to overflow and that water finally reached to reservoir pond for effective sedimentation. Ponds were initially prepared by drying, tilting (to remove the pests and predators and oxidize the bottom soil) and liming to correct the pH. Inorganic fertilizers such as urea and triple super phosphate were applied to develop the natural food organisms. Crab fencing and bird netting were done, before pumping water in to the ponds, to prevent the cross contaminations. The filter bags were checked for their correctness then the pumping was done to the entire ponds. After filling, water kept stranded for one day for settlement of sediments. Subsequently the water was chlorinated to the concentration of 60 ppm, after the chlorination the excess chlorine was neutralized by dechlorination process for 72 hours. After the dechlorination, fertilizer and Probiotic application was done for enhancing the water quality. The blooming of algal culture was noticed slowly in the ponds.

The *L. vannamei* seeds ($14^{th}$ post larval stage) purchased from CP Aquaculture India Private Ltd, hatchery Gudur, where the seeds were checked and confirmed negative result for the white spot syndrome virus (WSSV) infection by the polymerase chain reaction (PCR assay) test. The seeds were transported in oxygenated double-layered polythene bags with crushed ice packs between inner and outer covers of the bag and the entire set up was packed in a carton. The seeds were brought to the farm site and bags were allowed to float in the pond, in which the shrimp were to be stocked, acclimatized for a salinity level of 14 ppt and for other water quality parameters. The two ponds were managed identically during production cycles. The shrimps were fed with commercial pellet feed diet to which Probiotic, vitamin C, immune enhancers, and molasses were added as enhancers. Water probiotic were applied to the water weekly. No water exchange was done until the harvest.

Temperature, salinity, and pH values were measured twice a day (at 8:00 am and 6:00 pm) using a refractometer and pH pen, respectively. Dissolved oxygen (DO) was measured daily at 6:00 pm and at 1:00 am using a DO meter. The water level was measured by using a standard scale with cm marking. The light transparency of water was measured by using a secchi disc.

Cast net was used to measure the growth rate of shrimps. The first sampling was taken after $30^{th}$ days of culture (DOC) and number of individuals and the average body weights (ABW) were recorded in each sampling. Sampling was regularly performed for every ten days until the last harvest.

**Results and Discussion**. Water quality conditions in the ponds during the study period showed a normal range of fluctuations. Water temperature fluctuated between 24.0°C to 28.0°C, dissolved oxygen concentration fluctuated between 7.0 mg $l^{-1}$ at 6.00 pm and 10.0 mg $l^{-1}$ at 1.00 am, with average concentration of greater than 4.5 mg $l^{-1}$ most of the time, salinity value fluctuated between 14 to 17‰, water pH value fluctuated between 7.6 to 8.1. The sampling was done for every ten days to know the animal health and body weight. The $70^{th}$ DOC the both ponds (pond 1 and Pond 2) were affected by WSSV infection, the shrimps got affected due to bird dropping of (cross contamination through bird) infected shrimps from the neighboring *P. monodon* shrimp farm (non bio secured farm). The feed intake was aggressively increased for two meals and dropped sharply from the third meals of the first day of infection. The infected shrimps were found in check tray as well as side of pond dyke. Immediately the shrimps were collected in both ponds and checked for WSSV infection through PCR test at private laboratory.

The PCR results showed that the shrimps infected with WSSV infection in pond 2 showed high mortality than pond 1 because of high density. The farm management started harvesting shrimps of both ponds because of high mortality. Pond 1 shrimps were in 14 gram (ABW) size with 72% survival and yield was 6048 kg. Pond 2 shrimps were in 15 gram (ABW) size with 86 % survival and yield was 15,480 kg (Table 1). WSSV as sole pathogen may cause disease and mortality 100% within 2-10 days after the onset of symptoms (Lightner 1996, Xu et al 2006). The present study also showed the mortality ratio around 25% in pond 1 and 12% in pond 2 with in one day of infection. The ability of shrimp to grow with WSSV for relatively long periods under good rearing conditions has been previously reported (Tsai et al 1999, Khadijah et al 2003) (Fig. 1 and Fig. 2). In present study, condition of the pond and water quality are quiet good even though the infection ratio is fast due to stress of high stocking density.

Table 1

Farm details

| | *Pond 1* | *Pond 2* |
|---|---|---|
| Water Spread Area | 0.96 ha | 2.4 ha |
| Initial stocking | 0.6 million | 1.2 million |
| Date of stocking | 09/09/2010 | 09/09/2010 |
| Stocking density | 62.5/m² | 50/ m² |
| Date of harvest | 22/11/10 | 23/11/10 |
| ABW | 14 g | 15 g |
| Harvest DOC | 74 | 75 |
| Survival | 72% | 86% |
| Production (kg) | 6048 | 15,480 |
| Production/ha (Kg) | 6300 | 6450 |

**Conclusions**. The present study strongly recommends to every shrimp farmers to go for bio secure measures like bird fencing and crab fencing as precautionary methods to avoid cross contamination of WSSV infection from other farms.

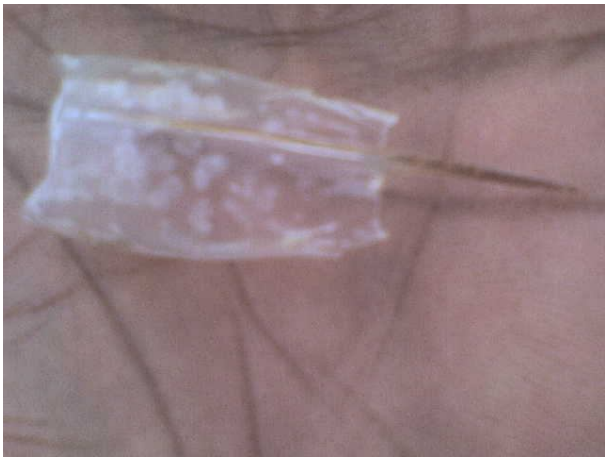

Figure 1. White spot on the carapace.

Figure 2. WSSV infected *L. vannamei.*

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