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Efficacy of Formalin for the Control of White Spot Syndrome Virus Infection in Black Tiger Shrimp (*Penaeus monodon*)

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ABSTRACT

White Spot Syndrome Virus (WSSV) is a virus found in invertebrates which is one of the leading causes of disease in commercially raised shrimp. The main method of controlling the disease is to prevent infections. This study found that formalin was effective in controlling WSSV in water. Concentrations of 100, 150 and 200 ppm of formalin could reduce the infection rate in shrimp that ate WSSV-infected shrimp. The survival rates for shrimp bathed in 100, 150 and 200 ppm formalin solutions were 33.33%, 30.00%, and 16.67%, respectively, compared to the control populations that were not exposed to formalin. Histopathological studies of shrimp tissue showed that shrimp infected with WSSV demonstrated histopathological changes within 24 hours even though on the outside they still looked normal. One method for controlling WSSV was to bathe post larvae in a 100 ppm formalin solution for 30 minutes before weeding out the weakest ones and putting the rest into the raising pond.

Key words: *Penaeus monodon*, black tiger shrimp, formalin, white spot syndrome virus

INTRODUCTION

White Spot Syndrome virus (WSSV) has caused much damage to shrimp farmers in Asia (Lo *et al*., 1996; Tapay *et al*., 1997). This is because it is easily contagious through water or other carriers and can be found in post larvae (Limsuwan, 1997). Most shrimps infected with WSSV have white spots on their cuticle (Wongteerasupaya *et al*., 1995). One reasonably effective and popular method of preventing WSSV in black tiger shrimp is preventing infections by eliminating carriers in the water in the shrimp pond before stocking the shrimp. This is usually accomplished by filtering the water or using chemicals to get rid of the carriers and then letting the water rest for at least 5 – 7 days before stocking post larvae in the growout ponds. However, even when this method is used, infections of WSSV can sometimes occur later after the shrimp have been introduced to the pond. This is because the virus came in with the post larvae. Another method of preventing WSSV that ought to be effective is to change the shrimp raising method from an open system with frequent water changes to a closed or semi-closed method with little or no water replacement, along with killing the virus and carriers before introducing the shrimp. This study was undertaken to study the feasibility of using formalin for controlling the spread of WSSV in black tiger shrimp. Formalin was chosen because it was a chemical widely used and the Fisheries Department of Thailand and many other countries had approved its use for raising aquatic animals. The results of could be used to form recommendations for shrimp farmers on how to

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reduce the severity of the WSSV disease.

MATERIALS AND METHODS

Preparation of WSSV for the experiments

Pure WSSV culture was obtained from the Aquatic Animal Health Research Institute. Department of Fisheries, Kasetsart University Campus, Bangkok, Thailand.

The WSSV stock in K199 virus medium (stored at –75° C) was prepared in the appropriate concentration before being injected into shrimp. Infected shrimp were detected by using the polymerase chain reaction (PCR) method to confirm the present of WSSV.

1.1 Ten-fold dilutions were made at the proportions of 1:10, $1:10^2$, $1:10^3$, $1:10^4$ and $1:10^5$

1.2 Two-fold dilutions were made between $1:10^4$ and $1:10^5$ as follows: $1:10^4$, 1:2, 1:4, 1:8 and $1:10^5$

Studying the most appropriate formalin concentration for preventing WSSV in shrimp

Normal shrimps weighing 3-5 grams were injected with WSSV. When they began to show symptoms of infection they were refrigerated and fed to normal shrimps for 3 hours. Then the samples were divided into groups of 30 shrimp as follows:

Group 1, 2, 3 were treated with 100 ppm, 150 ppm and 200 ppm formalin solution respectively for 30 minutes; Group 4 was negative control (infected shrimp) treated with water; Group 5 was positive control (uninfected shrimp) and then taken out into 3 clean water containers, each containing 10 shrimps.

Shrimps were observed for 7 days and the survival rates were recorded and analysed using analysis of variance and Duncan's multiple range tests . Histopathological studies were made on samples of shrimp before infection, 3, 6, 12, 24, 48, 72 and 96 hours post infection.

RESULTS AND DISCUSSIONS

Studying the most appropriate formalin concentration for preventing WSSV in shrimp found that there was a highly significant by statistical difference of 99% confidence rate between the formalin-treated group and the group that was not treated with formalin (Table 1). A

Table 1 Survival rates of black tiger shrimp eating shrimp infected with WSSV disease and being bathed in formalin solutions of varying concentrations.

Day	Survival rates (number of shrimp)				
	Group 1 formalin 100 ppm	Group 2 formalin 150 ppm	Group 3 formalin 200 ppm	Group 4 Not bathed formalin	Group 5 Not imfection with WSSV
1	10.0 ± 0.00	10.0 ± 0.00	10.0 ± 0.00	10.0 ± 0.00	10.0 ± 0.00
2	10.0 ± 0.00	10.0 ± 0.00	9.67 ± 0.577	10.0 ± 0.00	10.0 ± 0.00
3	9.0 ± 1.00	8.33 ± 0.577	8.33 ± 1.528	6.67 ± 1.528	10.0 ± 0.00
4	4.67 ± 0.577	4.67 ± 1.528	4.0 ± 1.00	2.67 ± 1.155	10.0 ± 0.00
5	4.0 ± 0.00	3.67 ± 0.577	3.0 ± 1.00	1.33 ± 0.577	10.0 ± 0.00
6	3.67 ± 0.577	3.33 ± 0.577	2.33 ± 1.528	0.00 ± 0.00	10.0 ± 0.00
7	3.33 ± 0.577 ^{c}	3.0 ± 0.000 ^{c}	1.67 ± 0.577 ^{b}	0.00 ± 0.577 ^{a}	10.0 ± 0.00
Total	33.33%	30.00%	16.67%	0%	100%

Note: The letters in superscript refer to statistical differences between the experimental groups as determined by DMRT.

comparison of the groups bathed in 100, 150 and 200 ppm formalin solutions showed that there was no statistical difference between the 100 ppm group and the 150 ppm group. The survival rates for these groups 7 days post infection were 33.33 and 30%, respectively. However, a comparison of the survival rate of the shrimp in these two groups with the group bathed in 200 ppm formalin solution showed a highly significant statistical difference at a confidence rate of 99% while survival rate of shrimp in the group bathed in 200 ppm formalin solution was only 16.67%.

It was also found that formalin in concentrations of 100, 150, and 200 ppm could halt the spread of WSSV. Pratanpipat *et al*. (1996) reported that formalin in a concentration of 70 ppm could kill WSSV in water. Therefore, the concentration used in this experiment was strong enough to kill all the WSSV in the water. Any further spread of the virus in this experiment could only occur from healthy shrimps eating shrimps with disease. A comparison of the effectiveness of the 3 formalin concentrations in stopping the spread of WSSV in shrimp fed with diseased shrimp showed that the concentrations of 100 ppm and 150 ppm were more effective in increasing the survival rate than 200 ppm. This might be because exposure to the strong formalin solution at a concentration of 200 ppm might stress the shrimp, resulting in mortalities of shrimp weakened from WSSV.

If a farmer can detect an outbreak of WSSV quickly enough, before many of the shrimp in the pond are infected, then the use of formalin to control the spread of the disease, combined with measures to quarantine or eliminate the shrimp that have already been infected, could be an effective method for solving the problem of WSSV outbreak. However, formalin at the concentrations of 100 – 150 ppm will also affect water quality because it will kill plankton in the shrimp pond. Formalin also reduces the dissolved the oxygen level, even to a level that is dangerous to the shrimp. Therefore, a method that could be more appropriate for administering formalin is to bathe post larvae 12-15 in a formalin solution of 100 – 150 ppm for 30 minutes before releasing them into the pond. Chanratchakool *et al*. (1998) reported that a formalin bath at a concentration of 100 – 200 ppm administered to post larvae after they were transported for 30 – 60 minutes, and before the stronger ones were picked out to be released in the pond, could reduce the problem of WSSV outbreak to a certain degree and did not affect the survival rate.

Regarding this study of the progression of histopathological changes in the tissues of black tiger shrimp infected with WSSV, it was found that histopathological changes were apparent in the shrimp 24 hours after they were exposed to the virus. At that stage the shrimp displayed no outward symptoms of the disease at all. The changed tissues were subcuticular epithelium, stomach lining, gills, hemopoietic tissue, lymphoid organ and the blood cells (Figure 1a,b). This was in agreement with the results of Flegel and Sriurairatana (1994) who reported that shrimp infected with WSSV would begin to show histopathological changes in 24 – 36 hours post infection, including nuclear hypertrophy, nuclear inclusions and necrosis of the affected tissues and cells. Wongteerasupaya *et al*. (1995) reported that in the early stages of infection nuclear hypertrophy would be visible in the cells developed from ectoderm and mesoderm, while in the next stage the nuclei would be entirely destroyed and empty spaces would be seen in the cells. Many cells would die. The results of the experiment suggested that histopathological studies of shrimp tissues could be another efficient and cost effective method of inspecting for WSSV, because irregularities could be detected in just 24 hours postinfection in still clinically normal shrimp.

CONCLUSION

Formalin at the concentration of 100 ppm

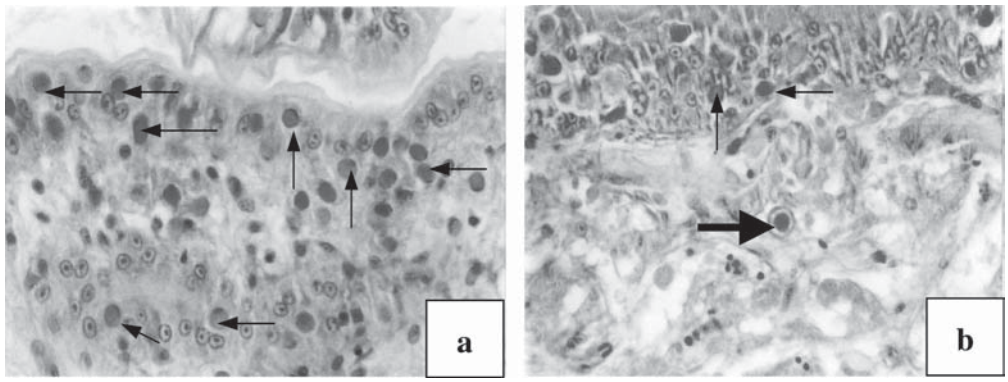

Figure 1 (a,b) Several cells with WSSV showing nuclear hypertrophy (small arrow) and adjacent cell with eosinophilic intranuclear inclusions (Cowdry type A inclusion; large arrow) (Davidson's; H&E; x400)

could reduce the mortality from infected shrimp with WSSV. In order to prevent WSSV outbreak in grow out pond during the culture period, shrimp farmer should bathe post larvae in a 100 ppm formalin for 30 minutes before stocking into culture pond.

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LITERATURE CITED

Chanratchakool, P. and C. Limsuwan. 1998. Application of PCR and formalin treatment to prevent white spot disease in shrimp, pp. 287-289. *In* T.W. Flegel (ed.). **Advances in Shrimp Biotechnology**. National Center for Genetic Engineering and Biotechnology. Bangkok.

Flegel, T.W. and S. Sriurairatana. 1994. Shrimp health management : an environmental approach, pp.1-48. *In* **Diseases in Aquaculture**, Malaysian Fisheries Society, Publication No.8.

Limsuwan, C. 1997. Reducing the effects of white-spot baculovirus using PCR screening and stressors. **The AAHRI Newsletter** 6(1): 1-2.

Lo, C.F., C.H. Ho, C.H. Chen, S.E. Peng, Y.T. Chen, C.M. Chou, P.Y. Yeh, C.J. Huang, H.Y. Chou, C.H. Wang and G.H. Kou. 1996. Detection of baculovirus associated with white spot syndrom (WSBV) in penaeid shrimps using polymerase chain reaction. **Dis. Aquat. Org**. 25:133-141.

Pratanpipat, P., C. Nithimethachoke, A. Akarajamon, G. Nash, B. Withyachumnam-kul, S. Thammasart and C. Lohawattanakul. 1996. p. 318 *In* R. Le Roy Creswell (ed.). The Efficacy of Formalin for Disinfecting of Systemic Ectodermal and Mesodermal Baculovirus. **World Aquaculture**'96.

Tapay, L.M., Y. Lu., R.B. Gose, E.C. Nadala, J.A. Brock and P.C. Loh. 1997. Development of an *in vitro* quantal assay in primary cell cultures for non-occluded baculo-like virus of penaeid shrimp. **J Virol Methods** 64:37-41.

Wongteerasupaya, C., J.E. Vickers, S. Sriurairatana, G. Nash, A. Akarajamon, V. Bonsang, S. Panyim, A. Tassanakajon, B. Withyachumnamkul and T.W. Flegel. 1995. A non-occluded, systemic baculovirus that occurs in the cells of ectodermal and mesodermal origin and causes high mortality in the black tiger prawn, *Penaeus monodon*. **Dis.Aquat.Org**. 21: 69-77.