Application of *Clinacanthus nutans* for control of viral disease in black tiger prawn (*Penaeus monodon*)

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KEY WORDS: Yellow head disease, whit spot syndrome, black tiger, medical herb, biological control

INTRODUCTION

Clinacanthus nutans is a common Thai herb grown in many parts of Thailand. This herb is known to be a traditional medicine for treatment of diseases caused by Herpes simplex and Xoster virus in human being. A preliminary in vitro experiment on using *C. nutans* to inhibit the viral pathogens isolated from diseased prawn was shown to be effective, which was reported by Direkbussarakom *et al.*¹⁾ Therefore, development on the method of using this medical herb for control of the yellow head baculovirus (YHV) and systemic ectodermal and mesodermal baculovirus (SEMBV) which cause mass mortality to black tiger prawn (*Penaeus monodon*), in several regions of the world, was carried out. The research aims to develop an appropriate method on application of this herb for the control of YHV and SEMBV. Side effect of the herb to the prawn itself as well as the impact to the culture environment after treatment by the herb was also studied.

MATERIALS AND METHODS

Application of *C. nutans* for control of YHV and SEMBV

Oral administration: extracts of *C. nutans* were mixed with commercial pellet feed at 0, 0.01, 0.1 and 1 g/kg feed. Each pellet set was fed to 400 prawn samples of the average size of 20 g which were reared separately in 4 tanks. After oral administration for 7, 14, 21 and 28 days, the prawns were transferred from each tank into 2 sets of aquarium. Each aquarium contained 18 samples. Artificial infection of the prawn by YHV and SEMBV via water borne route was performed for each sample set.

Bathing administration: Herb extracts were dissolved at 0.1 g/l seawater and standing at room temperature. Each group of prawn sample with the average size of 20 g was infected using intramuscular injection with 0.2 ml suspension of YHV and SEMBV, then bathing administration of the dissolved herb was conducted for 1 and 9 h, respectively.

All trials were conducted in 3 replications and observation on the relative percent survival was made for 14 days after challenge. The trials were repeated 2 times using the same method.

Study on side effect of *C. nutans*

Side effect to the prawn samples: Growth rate of the prawn samples was determined based on length and weight of 30 prawn randomly sampled from each group after receiving the herb at 0, 0.01, 0.1 and 1 g/kg feed for 28 days. Both total bacterial counts and those for *Vibrio* spp. as well as the bacterial composition in the hepatopancreas, hemolymph and intestine of the treated and controlled groups were determined based on Ruangpan *et al.*²⁾ Effect on immune system was evaluated by agglutinin titer^{3,4)} and phagocytic activity⁵⁾. Histological effects were observed in hepatopancreas, stomach and mid gut using the method applied from Bell & Lightner⁶⁾.

Side effect to the environment: Ten samples of prawn pond water were obtained from different farms located in different areas.

Each sample was added with herb extract to set the concentration at 0, 0.1 and 1 mg/l and stand under temperature between 27-29 °C for 28 days. Three replications were made for each trial. Flora and composition of total and *Vibrio* bacteria were studied based on Ruangpan⁷⁾ method at 7 days interval. Effect on the growth rate of plankton was studied using the representative species including *Chlorella*, *Tetraselmis*, *Skeletonema costatum*, *Chaetoceros gracilis*, *Spirulina platensis*, *Brachionus rotundiformis* and *Artemia* sp. Flora and composition of plankton were also studied. Quality of the water samples including chemical oxygen demand (COD) biochemical oxygen demand (BOD), total organic carbon (TOC), total Kjeldahl nitrogen (TKN), pH and alkalinity were daily checked for 7 days using Strickland & Parsons⁸⁾ and APHA, AWWA and WPCP⁹⁾. Statistical analysis of the data in all trials was done by ANOVA program.

RESULTS

The relative percent survival (RPS) of the prawn in control and administered group with different concentrations of herb extract prior to challenge with YHV and SEMBV are shown in Table 1. The optimal concentration of herb extract which revealed most effective in controlling viral infection by oral administration was 0.1 g/kg for YHV and 1 g/kg feed for SEMBV and bathing for administration was 0.1 mg/l for YHV. The average RPSs of prawn samples administered by feeding were 56.1, 30.8, 31.6 and 24.2% for YHV and 13.8, 33.4, 30.4 and 25.0% for SEMBV after receiving the herb for 7, 14, 21 and 28 days, respectively. Bathing administration for 1 h in dissolved herb could control 60% of YHV infected prawn.

Statistical evaluation based on ANOVA analysis ($P > 0.05$) showed that no side effect in prawn's growth rate, immune system, tissue of the hepatopancreas, stomach and intestine as well as the flora and composition of the bacteria in hepatopancreas, hemolymph and intestine. No changes in the values of the water qualities, flora and composition of plankton and bacteria was observed, when compared among the control and treated samples even 10 times higher than the effective concentration of herb extract which recommended for the control of the viral pathogens in our study.

Table 1 Relative percent survival of the prawn administrated with herb extract prior to challenge with YHV and SEMBV

Oral Admin.	Average % RPS					
	<i>C. nutans</i> concentration (g/kg feed)					
	0.01	0.1	1	0.01	0.1	1
	YHV			SEMBV		
7 days	43.9	56.1	9.8	-3.5	37.9	13.8
14 days	23.1	30.8	12.8	12.1	9.1	33.4
21 days	18.4	31.6	10.5	34.8	17.4	30.4
28 days	18.2	24.2	36.4	16.7	-2.5	25
0.1 mg/l seawater						
Bathing	YHV			SEMBV		
1 h dissolved herb	60			Not tested		
9 h dissolved herb	15.7			Not tested		

DISCUSSION

Application of *C. nutans* to inhibit YHV infection was preliminarily tested by exposure of the pathogen to herb extracts in the test tube¹⁾. The effective inhibitory concentration was reported as 1 µg/ml seawater. The results of our studies on oral administration showed that the herb extract at 0.1 g/kg feed was the effective concentration for control of YHV, where as 1g/kg feed was effective for SEMBV. For bathing administration, 0.1 mg/l seawater standing under room temperature for 1 h was the optimal level for control of YHV and gave 60% RPS. According to the statistical analysis of the data obtained from the samples receiving oral administration for 7, 14, 21 and 28 days which showed almost the same level of RPS. This may reveal that using *C. nutans* for the control of the viral pathogens is

necessary to apply continuously for a long period. Since there was no side effect of the herb to the prawn and pond environment even when treated the herb extract 10 times higher than the effective dose. It was suggested that *C. nutans* is an effective and safe medical herb for the control of YHV and SEMBV in black tiger prawn. However, further studies are needed to prove the herb's efficacy to eliminate YHV and SEMBV in prawn brood stocks and postlarvae, the latter being the most susceptible stages for transmission of the disease.

ACKNOWLEDGEMENT

Appreciation was given to "The Thailand Research Fund" which supported our study.

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Leaf of *C. nutans*

Flower of *C. nutans*