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RSEARCH PAPER

Immunomodulatory effect of *Sargassum wightii* on *Penaeus monodon* (Fab.)

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ABSTRACT

Immunomodulatory efficacy of methanolic extract of seaweed, *Sargassum wightii* was assessed on shrimp, *Penaeus monodon*. Three diets were prepared by supplementing seaweed, *S. wightii* extract at the rate of 100, 200 and 300 mg/100g feed. The conventional feed ingredients such as fish meal, soymeal, groundnut oil cake, rice bran and tapioca powder were used as the basal feed. Feed additives such as vitamin and mineral mix, cod liver oil, stickon, chromic oxide and NaCl were also added in the feed. A diet devoid of seaweed extract was also prepared and used as a control feed. The efficacy of these tests and control diets were evaluated on *Penaeus monodon* stocked at the rate of 15 Nos/m^3 in an outdoor culture 1 tonne capacity FRP tanks for a period of 90 days.The maximum survival of 96.66% was recorded in *P. monodon* fed with 100 mg and 300mg seaweed extract added diet against the minimum survival of 83.33% registered in those fed with control diet. The growth parameters such as food consumption, SGR and FCR were more in *P. monodon* fed with seaweed extract added diets. The total haemocyte count was also high in *P. monodon* received seaweed extract added diets. Similar variation was also noticed for bacterial clearance and phenol oxidase activity.

Key words : *Penaeus monodon*, Immunomodulation, Methanolic extract, Growth responses

Penaeus monodon is the most preferable species for coastal aquaculture due to its large size, adaptability to varying salinities, high demand, etc. (Pillai *et al.*, 1996). Knowledge on *P. monodon* nutrition has increased greatly in recent years. However, the shrimp industry had always been affected by infectious diseases, mainly of bacterial and viral etiology (Lightner *et al.*, 1983; Kroll *et al.*, 1991; Hasson *et al.*, 1995) causing great production loss. The sustainability of shrimp industry depends largely on disease control and health status of shrimp. Immune system is used as a tool to assess the shrimp health (Bachere *et al.*, 1995). In penaeid shrimp bacteria, *Vibrio alginolyticus*, *Vibrio harveyi* and *Photobacterium damselae* sub sp. are considered to be secondary and opportunistic pathogens, and have been demonstrated to cause disease outbreaks which is associated with poor environmental conditions (Lee and Chen, 1994; Lee *et al.*, 1996; Liu *et al.*, 1996). Shrimp diseases have also been reported to be associated with the increase of *Vibrio* population in culture pond waters (Sung *et al.*, 2001). Therefore, the health status of shrimp and its immunity are of primary concern. Since shrimps lack an adaptive immune system, they rely on non-specific immune responses against microbial invasions. Haemocytes play a central role in crustacean immune defence in removing the foreign particles by phagocytosis and would healing.

The prophenol oxidase (propo) system is an important component of defence system in decapod crustaceans. Its activation elicits defence reaction such as phagocytosis and melanin synthesis. Melanin and its reactive intermediates have shown to be fungistatic (Soderhall and Ajaxon, 1982; Persson *et al.*, 1987). Phenol oxidase (PO) is the terminal enzyme in the prophenoloxidase system which is activated by minute amounts of microbial cell wall components such as lipopolysaccharides (LPS).

Certain seaweeds are also having anti-microbial activity against a number of pathogenic and non-pathogenic bacteria (BoomaKasthuri, 1998). Hot water extract from seaweed brown algae including *Undaria pinnatifida* and *Sargassum autunnale* have been reported to increase the resistance of common carp against *Edwardsiella tarda* (Fujiki *et al.,* 1992). Sodium alginate extracted from brown algae, *Undaria pinnatifida* and *Lessonia nigrescens* have been reported to increase the resistance of *L. vannamei* against *Vibrio alginolyticus* (Cheng *et al.*, 2004; Cheng *et al.*, 2005). Reports have been observed that the hot water extract of brown seaweed, *Sargassum duplicatum* also had increased the immune resistance in white shrimp, *Litopenaeus vannamei* (Jiann-chu Chen *et al.*, 2006).

The purpose of the present study is to evaluate the efficiency of methanolic extract of seaweed, *Sargassum wightii* on growth and immune responses in shrimp, *Penaeus monodon*.

MATERIALS AND METHODS

Penaeus monodon:

Penaeus monodon post-larvae (PL20) were obtained from a commercial hatchery Matsyafed, Kollam, Kerala state. The collected seeds were transported to the laboratory by oxygenated polythene bags and acclimatized in well aerated seawater in one tonne capacity FRP tank. The post-larvae were fed with *Artemia nauplii* for two days and subsequently fed with artificial feed for a week. After acclimatization, the healthy post-larvae were selected and starved for 24 hours prior to the start of experiment in order to evacuate their gut contents. The initial weight of the post-larvae was measured and they were grouped into four (C, F_1, F_2 and F_3) each consisting of 30 individuals. Then they were reared in four one-tonne capacity FRP tanks containing 800 l of seawater at a constant salinity of 25 ppt, with pH 8.12 to 8.89 and temperature of 30.0 ± 1.5^{0}C for a period of 90 days. By doing daily water exchange of 20 to 30%, uniform water quality parameters were maintained in control and experimental tanks.

Seaweed extract :

The seaweed, *Sargassum wightii* was collected during low water spring tides from gulf of Mannar at Mandapam. After collection, they were washed thoroughly in seawater and followed by freshwater to remove the debris and shade dried for 10 days. Dried seaweed was powdered and extracted with methanol by using Soxhlet apparatus. The extract was then condensed, evaporated and dried to powdered form.

Feed formulation and feeding :

By supplementing the seaweed powder at the rate of 100, 200 and 300 mg/100g feed, three test diets (F_1, F_2 and F_3) and a control diet (C) were prepared using conventional feed ingredients. The sieved ingredients were weighed and mixed thoroughly with sufficient quantity of water and made into a dough. The dough was steam boiled using a pressure cooker for 15minutes. Cod liver oil, vitamin and mineral mix and chromic oxide (500 mg) were added. Control and experimental diets were then extracted in the form of noodles using a pelletizer having perforation diameter of 3 mm and then they were sun dried to reduce the moisture content and broken manually to a size of about 1 cm and stored. The shrimps were fed daily *ad libitum* by check tray method in control tank and experimental tanks, respectively.

Vibrio harveyi :

The bacterium, *Vibrio harveyi* was obtained from Marine Biotechnology Loboratary, Vizhinjam Research Centre of Central Marine Fisheries, Vizhinjam, Kerala. For bacterial clearance test, 100µl suspension of 12 hrs culture were injected into the ventral sinus. After injection, the shrimps were kept in aerated plastic tanks containing 80 l of water for 180 min. Then 100µl of haemolymph was collected and diluted with sterile saline and spread on to triplicate TCBS agar plate. The samples were mixed with melted TCBS agar, poured into Petridishes and incubated at appropriate room temperature for 18 hrs. Number of bacterial colonies per plate were counted and divided by the volume of haemolymph, extracted to determine the number of colony forming units (CFU) per milliliter of haemolymph (Adams, 1991).

Shrimp and haemolymph collection :

Penaeus monodon (both control and experimental) grown for 90 days were collected and the haemolymph were obtained from the ventral sinus using a 1 ml syringe having 25 gauge needle and filled with 0.2 ml of 12.5% of sodium citrate as an anticoagulant. In each experiment, haemolymph was collected from 3 shrimps, pooled, mixed well and stored in Eppendorff tube and kept at low temperature for further analysis.

Total haemocyte count :

Total haemocyte count in the test samples was carried out by using Naubaeur Haemocytometer with Gentain violet stain. Haemocytes found on the entire corner of 1 mm squares were counted following the formula of Jones (1962).

Phenol oxidase assay :

Phenol oxidase assay was done (100 µl of haemolymph) spectrophotometrically by recording the formation of dopachrome formed from L-dihydroxy phenyl alanine (L-Dopa) following the method of Preston and Taylor (1970).

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

***Survival* :**

The survival of *P. monodon* fed on control and experimental diets showed marked variation and it was maximum (96.66%) for those shrimps received diets containing 100 and 300 mg of seaweed extract. Shrimps fed with 200 mg seaweed extract added diets the survival was 93.33%, where as the minimum survival of 83.33% was recorded in control diet fed shrimps (Fig. 1).

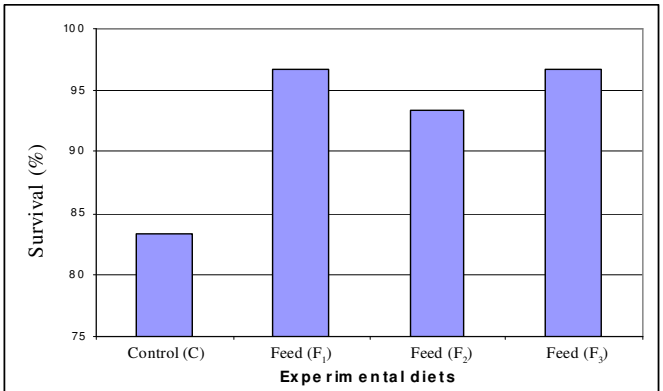

Fig. 1 : Survival (%) of Penaeus monodon fed with control (C) and seaweed extract added diets (F_1 - F_3) during the experimental period

Growth responses :

The results on growth responses showed that the maximum production of 7.35 g was registered in *P. monodon* fed on F_3 diet and in other diets fed shrimps it was low. The food conversion efficiency ranged from 63.29% to 82.64% with the maximum in F_3 diet fed shrimps. The food conversion ratio in control and experimental diets fed shrimps ranged from 1.21 to 1.58 (Table 1).

Table 1 : Overall growth responses of *P. monodon* fed with control (C) and seaweed extract added diets

Parameters	Growth responses			
	Control	Feed 1	Feed 2	Feed 3
Initial weight (g)	0.066	0.010	0.006	0.007
Final weight (g)	6.50	7.32	4.65	7.35
Production (g)	6.434	7.31	4.644	7.343
Food consumed (g)	10.165	9.66	6.424	8.885
Food Conversion Efficiency (%)	63.29	75.673	72.29	82.645
Absolute Growth Rate (g/body wt/day)	0.071	0.081	0.051	0.081
Specific Growth Rate (%)	5.1	7.3	7.39	7.3
Food Conversion Ratio	1.58	1.32	1.38	1.21

Each values is the mean of three individual estimates

Non-specific immunological parameters :

Non specific immunological parameters such as total haemocyte count, bacterial clearance and phenol oxidase assay were done at the end of the experimental period in *P. monodon* fed with control and experimental diets. The total haemocyte count showed an increasing trend in the seaweed supplemented diet fed shrimps and the maximum value of 920 ± 29.0 cells/mm^3 was recorded in F_3 diet fed groups. In control diet fed shrimps a minimum value of 642 ± 22.0 cells/mm^3 was noticed (Table 2).

Table 2 : Total haemocyte count in *P. monodon* fed on control (C) and seaweed extract added diets (F_1 to F_3) at the end of the experimental duration

Experiment	Total haemocyte counts (cells/mm^3)
Control (C)	642 ± 22.0
Feed (F_1)	880 ± 35.0
Feed (F_2)	642 ± 26.0
Feed (F_3)	920 ± 29.0

Each value is the mean of three estimates

The bacterial clearance (%) was measured at the end of the experimental period both in control and experimental diet fed shrimp (F_1 – F_3), shaved a high value in *P. monodon* fed on experimental diets. On the other hand in *P. monodon* fed on control diet, the percentage clearance was low (43.28 %) (Fig. 2).

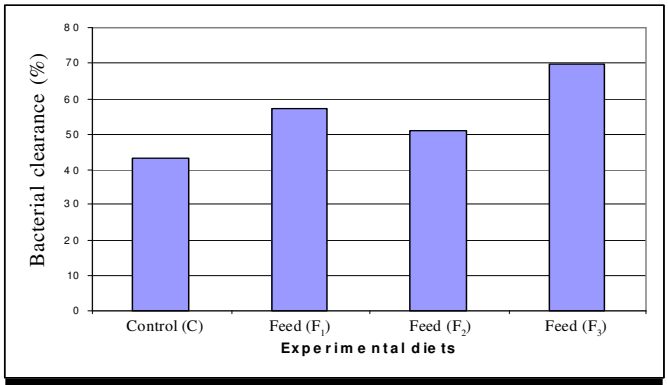

Fig. 2 : Bacterial clearance (%) of Penaeus monodon fed with control (C) and seaweed extract added diets (F_1 - F_3)

Phenol oxidase activity :

Phenol oxidase activity in the haemolymph of *P. monodon* fed on control (C) and experimental diets (F_1 – F_3) varied much. A minimum phenol oxidase activity of 0.06 OD was recorded for those of shrimps fed on control diet. But the maximum phenol oxidase activity of 0.11 OD was recorded in shrimp fed on F1 diet. In other experimental diet fed shrimps, the phenol oxidase value recorded was high compared with the value registered for those shrimps on fed control diet and the values observed were 0.08 OD in F_2 and 0.09 OD in F_3 diet fed shrimps, respectively (Fig. 3).

Diet studies showed that marine natural products were found effective in supporting larval growth and survival (Devi, 1995). In the present study, addition of seaweed extract in the diet enhanced the survival of *P. monodon*. The survival of *P. monodon* fed with seaweed extract added diets (F_1 to F_3) was high (93.33% to 96.66%) against the low value (83.33%) recorded for those shrimps that received control diets. Similar enhancement in survival

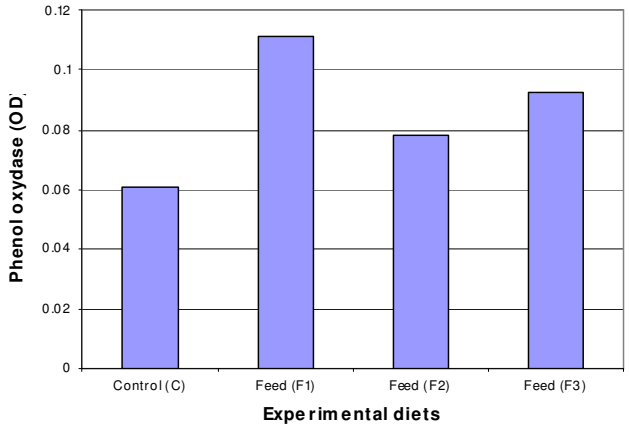

Fig. 3 : Phenol oxidase activity (OD) in the haemolymph of *P. monodon* fed with control (C) and seaweed extract added diets (F_1 - F_3)

of shrimp was also reported by earlier workers like Yamada *et al.* (1990). They reported that survival of 6.12 g shrimp fed with 100 ppm astaxanthin supplemented diet improved from 57.1% to 83.7% when compared to that of astaxanthin free diet.

Quite interestingly, addition of seaweed extract in the diet enhanced the growth performance of *P. monodon* in the present study. The maximum production (7.343 g) and FCR values (1.21) were recorded in *P. monodon* received diet containing 300 mg/100g feed. Shrimp fed with diet containing 100 mg seaweed extract / 100g feed also displayed the equal growth performance in bar with those received diets containing 300 mg seaweed extract / 100g feed. This present result is consistence with the earlier work of Sung *et al.* (1991). Boonyaratpalin *et al.* (1995) also reported that tiger shrimp fed on peptidoglycan (PG) supplement feed showed better growth and feed conversion rates than those fed in normal diets.

Logamble *et al.* (2000) reported the effect of *Ocimum sanctum* leaf extract on specific and non-specific immune response and disease resistance against *Aeromonas hydrophila* in *Oreochromis mossambicus*. Several polysaccharides extracted from marine algae enhanced non-specific immune system in shrimp. The administration of hot water extract of *Sargassum duplicatum* reported to increase the resistance of several fish and shrimp species against bacterial infections (Fujiki *et al.*, 1992 ; Cheng *et al.*, 2004; Cheng *et al.*, 2005). The oral administration of fucoidan extracted from brown alga, *Sargassum polycystum* had been reported to reduce the impact of white spot syndrome virus (WSSV) infection in tiger shrimp, *P. monodon* (Chotigeat *et al.*, 2004). In the present study also, *P. monodon* that received methanolic extract of *Sargassum wightii* as a dietary source of administration, showed enhanced resistance against *Vibrio harveyi*.

Total haemocyte counts (THC) provide an useful information as in assessing the physiological stat of crustaceans (Martin and Graves, 1985). Huxley (2002) reported that THC obtained from immunostimulants fed shrimps were higher over the control group. Results in the present study also indicated that shrimps fed on seaweed extract diets showed a high total haemocyte count compared to that of control. Also, the bacterial clearance was high in experimental diet fed *P. monodon* compared to that of control diet fed groups. This is obviously due to the enhanced non-specific immunity in *P. monodon* by crude bioactive extract from *S. wightii*.

In an *in vitro* study, laminarin extracted from brown alga, *Laminaria digitata* was reported to enhance the prophenol oxidsase (pro PO) system in brown shrimp, *Farfentepenaeus californiensis* (Hernandez *et al.*, 1996). It is known that *L. vannamei* injected with sodium alginate extracted from *Macrocystis pyrifera* had increased PO activity (Cheng *et al.*, 2004). In the present study also, *P. monodon* that received crude bioactive compound extracted from *S. wightii* through oral administration enhanced the phenol oxidase activity. From this study it is evident that the crude bioactive compound extracted from *S. wightii* activated non- specific immune system in *P. monodon*. Further studies on isolation and identification of specific compound are essential for understanding the mode of action and also for further applications.

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