Effect of hot-water extract of brown seaweed *Sargassum glaucescens* via immersion route on immune responses of *Fenneropenaeus indicus*

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Abstract

The development of shrimp aquaculture, in spite of its global necessity, is largely at stake as significant ecological and pathological problems are increasing in the vast majority of the shrimp producing countries. Shrimp immunology is a key element in establishing strategies for controlling diseases in shrimp aquaculture. The total haemocyte count (THC), differential haemocyte count (DHC), total plasma protein (TPP), Phagocytic activity (PA), bacterial clearance efficiency (BCE) and bactericidal activity (BE) were examined when the *F. indicus* shrimps (11.32±1.20 g) were immersed in seawater (39 ppt and 25 ± 1 °C) containing hot-water extracts of brown alga *Sargassum glaucescens* at 100, 300 and 500 mg/l. These parameters increased significantly ($p < 0.05$) when the shrimp were immersed in seawater containing hot-water extracts at 100 mg/l after 3h and 300 and 500 mg/l after 2 h. *F. indicus* shrimps that were immersed in hot-water extracts at 300 and 500 mg/l had increased phagocytic activity and clearance efficiency to *Vibrio spp.* after 2 hours. But bactericidal activity increased significantly after 1 hour in the same concentrations.

Keywords: *Fenneropenaeus indicus*; *Sargassum glaucescens*, Total haemocyte count, Differential haemocyte count, Total plasma protein, Phagocytic activity, Bacterial clearance efficiency

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Introduction

Immunostimulatory trace of some bacteria, glucans, peptidoglycans, lipopolysaccharides (LPS) (Sakai, 1999; Song and Huang, 1999; Smith et al. 2003) and other polysaccharides, schizophyllan extracted from the fungus *Schizophyllum commune*, scleroglucan extracted from the fungus *Sclerotium glucanicum*, and yeast glucan extracted from yeast *Saccharomyces cerevisiae* have been widely studied. The potency of these components to increase non-specific immune system has proved in Penaeid shrimp (Yano et al., 1991; Sakai, 1999; Song and Huang, 1999; Chang et al., 2000) have been widely studied in fish and crustaceans.

Immunostimulatory potency of hot-water extracts from several brown algae species including *Undaria pinnatifida* and *Sargassum autumnale* have been studied on common carp (*Cyprinus carpio*) against *Edwardsiella tarda* and yellow tail (*Seriola quinqueradiata*) against *Streptococcus* infection (Fujiki and Yano, 1997). Sodium alginate extracted from brown algae *U. pinnatifida* and *Lessonia nigrescens* have been reported to increase the resistance of *Litopenaeus vannamei* against *Vibrio alginolyticus* (Cheng et al., 2004, 2005). Some investigators evaluated immunostimulant effects of hot water extracts of brown algae on the shrimp immune system (Yeh et al., 2005; Balasubramanian et al., 2008). Shrimp culture industry has undergone great development since 1970 in the world, and shrimp farming industry with green tiger shrimp (*P. semisulcatus*), Indian white shrimp (*F. indicus*) and recently *L. vannamei* has been established in Iran more than 10 years ago. Disease outbreaks were associated with increases in the proportion of potentially pathogenic species in the *Vibrio* population of cultured pond waters (Song et al., 1993; Smith et al. 2003; Emadi et al., 2010). White spot disease outbreak in Iran from six years ago (Afsharnasab et al. 2006) motivated us to study on shrimp health and enhancement of shrimp immunity as a primary concern.

This study was undertaken to examine the immune response of *F. indicus* after treatment with hot-water extracts of *S. glaucescense*, a common brown seaweed which is distributed around the coastal area of Bushehr province in south of Iran. The shrimp were immersed in seawater containing hot-water extracts. Several immune parameters were examined following treatment with hot-water extracts of *S. glaucescense*, including total haemocyte count (THC), differential haemocyte count (DHC), total plasma protein (TPP), phagocytic activity (PA), bacterial clearance efficiency (BCE) and bactericidal efficiency (BE).

Materials and methods

Experimental design

Sargassum glaucescense was collected from around the coastal area of Bushehr province in the south of Iran in April and May 2005. Hot-water extracts of *S. glaucescense* was prepared based on the method of Fujiki et al. (1992). Briefly, the algal fronds were washed with water and dried naturally at room temperature. Ten g of the milled fronds was added to 300 ml

of deionized water, and the suspension was boiled for 3 h. The suspension was filtered through a nylon mesh, and the filtrated extract was lyophilized under reduced pressure. The hot-water extract at 1, 3 and 5 g was then dissolved in 100 ml distilled water and then mixed in 10 l sand-filtered seawater (39 ppt) to obtain final concentrations of 100, 300 and 500 mg/l, respectively. About 450 shrimp from the Helleh research station adjacent to the Bushehr city, Iran, were transferred to the Iran Shrimp Research Center (ISRC). Shrimps were placed in glass aquariums (155 l), and acclimated to room temperature (25±1°C) for two weeks. During the acclimation period, shrimps were fed three times daily with a formulated shrimp diet (Havoorash food Company, Bushehr, Iran). Only shrimps in the intermoult stage were used for the study (Liu et al., 2004). The molt stage was determined by examination of uropoda in which partial retraction of the epidermis could be distinguished (Robertson et al., 1987; Liu et al., 2004). Two studies were conducted, the first study was comprised of 10 shrimp each in triplicate for examining total haemocyte count (THC), differentional haemocyte count (DHC), phagocytic activity (PA) and total plasma protein (TPP), test and control groups. The second study was also comprised of 10 shrimp each in triplicate for the evaluation of bacterial clearance efficiency (BCE) and bactericidal efficiency (BE) to *V. harveyi*, test and control groups. The shrimps ranged from 10.12 g to 12.52 g, with an average of 11.32±1.20 g (mean ± SD, n=50) with no significant size differences among the treatments.

Chemicals and solutions

All chemicals were of analytical reagent grade. The glassware and solutions were pyrogen-free to avoid enzymatic interruption. The anticoagulant that avoids clotting after collection of haemolymph was prepared according to (Vargas-Albores et al., 1993): 450 mM NaCl, 10 mM KCl, 10 mM EDTA–Na_2, 10 mM HEPES, pH 7.3.

May-Grünwald-Giemsa staining Reagents (Houwen, 2000; Kakoolaki et al., 2010):

(1) Absolute methanol. (2) Staining solution I: 0.3 g May-Grünwald powder in 100 ml absolute methanol; must be left in a closed container at room temperature for 24 hours. It must be filtered before use. Staining solution II (Giemsa stain): 1 g Giemsa stain powder is dissolved in 66 ml glycerol and heated to 56°C for 90 to 120 minutes. After addition of 66 ml absolute methanol and thorough mixing, the solution is left at room temperature in a closed container. It must be filtered before use. (3) Buffer: Sörensen's buffer solution. The pH must be at 6.8 for the May-Grünwald-Giemsa stain.

The immune parameters of F. indicus immersed in aerated seawater containing hot-water extracts of S. glaucescense

For each examination of this study four concentrations were considered (0 (control), 100, 300, and 500 mg/l) and four exposure times (0, 1, 2, 3 and 4 h). Each treatment group was immersed in 20 liters seawater containing hot-water extracts at 0, 100, 300 and 500 mg/l, respectively. Ten shrimp for each treatment and time were used for the studies.

Specimen preparation

After 0, 1, 2, 3 and 4 h in the immersion test haemolymph (100 µl) was withdrawn from the ventral sinus of each shrimp into a 1 ml sterile syringe containing 0.9 ml precooled (4 °C) anticoagulant solution (prepared as mentioned before) and injected into the Eppendorf microfuge (solution A) (Vargas-Albores et al., 1993).

THC & DHC

A drop of solution A was placed on a haemocytometer and the THC was measured using a microscope (Nikon Photolab, Japan) with magnification of 400×. DHC was determined with the use of morphological criteria such as size and shape of cells and the difference of haemocyte refractivity using a light microscope (Le Moullac et al., 1998; Kakoolaki et al., 2010). Before observation under the microscope, cells must be stained by the May-Grünwald-Giemsa method. Therefore, two slides of each sample were prepared and after dryingdrying at room temperature they were stained as the Houwen (2000) method. In brief, they were then fixed for at least 30 seconds in absolute methanol; methanol was removed by tilting the slide or by simply removing from the fixing jar. Staining solution I freshly diluted with an equal part of the buffer was applied for 5 minutes on a horizontally positioned slide or in a jar. The slide was transferred from the jar without washing (or by removingremoving the staining solution by holding slide vertically) into staining solution II, that has been freshly diluted with 9 parts buffer for 10 to 15 minutes. The slide was transferred to a jar with buffer for 1 rinse after removing the stain. The slide was washed with ample water and then transferred to a jar containing water for 2 to 5 minutes. Then it was dried in a tilted position; do not blow dry. Mount a cover glass if desired. The remainder of the haemolymph mixture was used for subsequent tests.

Total plasma protein

Plasma protein concentrations were determined by the accepted Bradford's method (1976). All samples were measured in triplicate and calibrated against a bovine serum albumin (BSA) standard curve (0–200 mg/ml).

Phagocytic activity

Phagocytic activity was determined by the method of Jiang et al. (2004). Twenty five microlitres of solution A was placed on a dichromate-cleaned glass slide and incubated for 30 min at room temperature. Subsequently, 25 µl of *Staphylococcus aureus* at a concentration of 1×10^8 cells/ml was added to each solution A sample and the preparation was incubated for an additional 30 min. Then, each slide was washed with anticoagulant, fixed with 4% glutaraldehyde in the solution of anticoagulant for 1 min, rinsed in distilled water for 1 min, post fixed with 95% ethanol for 1 min, and air-dried. The slides were then stained with toluidine blue for 5 min and decolorized in running tap water. The number of ingested *S. aureus* and the number of haemocytes that have ingested *S. aureus* were counted from any of the 200 haemocytes observed using a light microscope at a magnification of ×1000 (Nikon, Photolab, Japan). The percentage

of phagocytosis was calculated as below (Weeks-Perkins et al., 1995):

- Phagocytosis percentage = (number of cells ingesting bacteria/number of cells observed) ×100.

Bactericidal activity

Vibrio harveyi was cultured in tryptic soy broth with 1,5% NaCl overnight at 25°C. Bacteria were collected by centrifugation and washed once in 2% sterile saline then diluted with saline to obtain the bacterial suspension at an optical density of 0.1 (540 nm). The haemolymph was prepared by centrifugation at 9,700 rpm for 20 minutes with anticoagulant. Then 100 µl of bacterial suspension was incubated with 100 µI of cell free haemolymph (plasma) (Song and Shiel, 1995). Samples were incubated in sterile microtubes for 3 hours at 25°C. Aliquots of 100 µl were taken from each microtube and spread onto thiosulphate citrate bile salt sucrose agar (TCBS) plates in order to count the colony forming units (cfu) (Liu et al. 2004**).** Positive controls were bacteria suspended in saline incubated in K-199 with l00µl anticoagulant.

Bacterial clearance efficiency

Following injection challenge with 1×10^5 cfu *Vibrio* spp. per shrimp and being kept for 1.0 h in a separate tank containing 40 l of water, 100µl haemolymph samples were taken from ventral sinus of shrimps and the samples were immediately added to 1.9 ml of precold (4 °C) sterile Van Harrevald's salt solution (VHS). Haemolymph (100µl) in VHS was spread onto TCBS agar plates for enumeration of numbers of total *Vihrio* spp. in haemolymph on TCBS plates which were counted after incubation time for I8 hours (Liu et al. 2004**)**.

Statistical analysis

Tukey's multiple comparison test was used by SPSS software to compare the significant differences among treatments. Before analysis, the data percentage (resistance studies) was normalized using an arc sin transformation. For statistically significant differences, it was required that $p < 0.05$.

Results

THC, DHC and TPP

THC of healthy *F. indicus* (control) in the experiment was within the range of 79.3×10^5 and 68.9×10^5 cells/ml. The THC of *F. indicus* that were immersed in hot-water extracts of *S. glaucescens* at 100 mg/l was significantly higher than control shrimp after 3 and 4h, and at 500 mg/l and 300 mg/l was significantly higher than control shrimp from the first hour to the end of the experiment. From 0 to 4 h of the experiment, the THC in 100 mg/ml, 300 mg/ml and 500 mg/ml treatments increased significantly to 112.4, 118.8 and 136.1 ($\times10^5$ cells/ml) ($p < 0.01$) respectively. The plasma protein concentrations of the control group was relatively stable from the beginning to the end of the experiment (69.16±10.86 and 77.16±12.35 mg/ml, $p > 0.05$) (Table 1). The plasma protein concentrations of shrimp were immersed in 100, 300 and 500 mg/ml hot water extracts of *S. glaucescens* which increased significantly after 2, 1 and 1 h respectively (Figs. 1 and 2).

Table 1: THC, DHC (×10^5 cells/ml) and TPP (mg/ml) of control and treated (immersed in 100, 300 and 500 mg/l hot water extracts of *S. glaucescens*) *F. indicus*

Haemolymph (×10^5 cells/ml)	Hot water extract of *S. glaucescens* (mg/l)			
	Control	100	300	500
THC	74.00±3.72	90.68±14.35	101.57±15.36	113.87±21.06
DHC				
• HC	53.5±4.0	61.7±7.0	70.9±10.1	79.3±13.9
• SGC	8.1±1.8	15.1±4.8	16.3±4.9	18.6±6.2
• GC	12.3±2.7	13.4±5.1	14.3±4.1	15.8±4.4
TPP (mg/ml)	74.15±9.62	94.34±15.81	102.03±16.39	101.50±14.64

HC (Hyalin Cells), SCG (Semi Granular Cells), GC (Granular Cells)

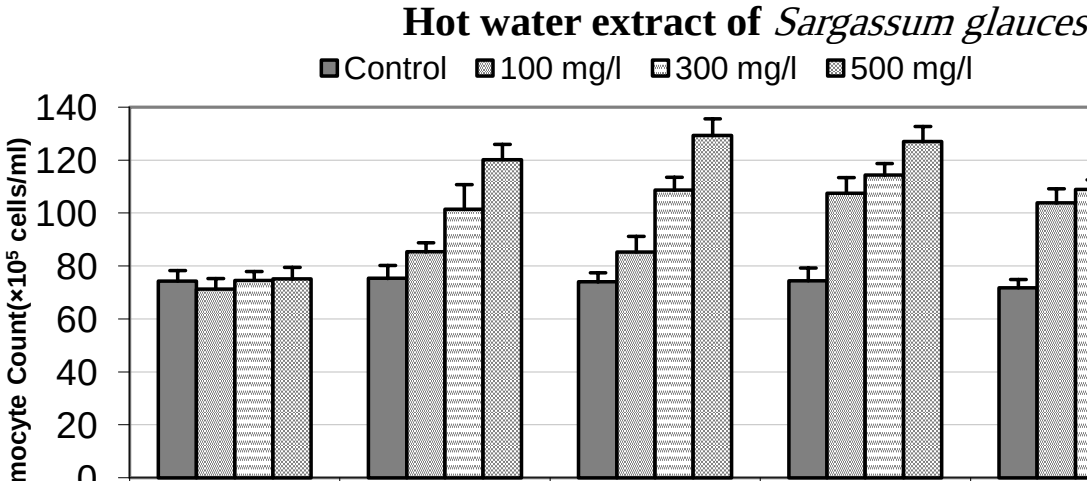

Figure 1: Mean (±SE) Total Haemocyte Count (×10^5 cells/ml) *F. indicus* immersed in seawater containing hot-water extracts of *S. glaucescens* in 500, 300 and 100 mg/l, and the control shrimp

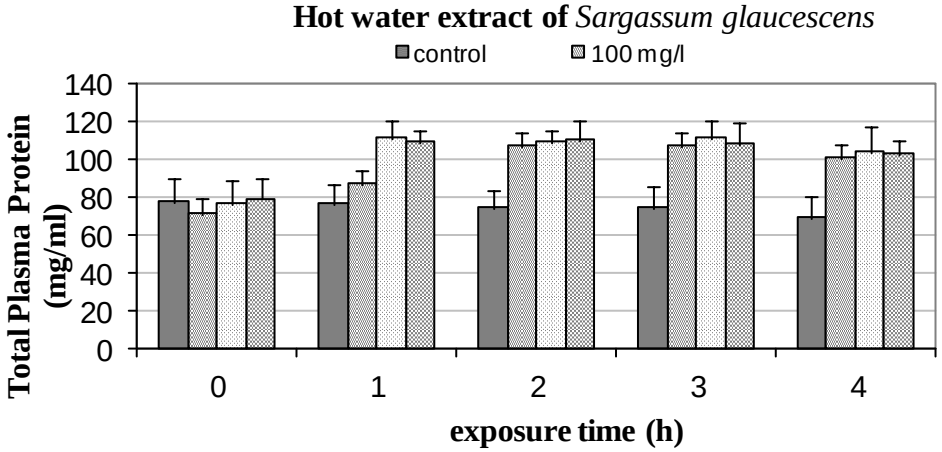

Figure 2: Mean (±SE) Total Plasma Protein (mg/ml) 0f *F. indicus* immersed in seawater containing hot-water extracts of *S. glaucescens* at 500, 300 and 100 mg/l, and the control shrimp.

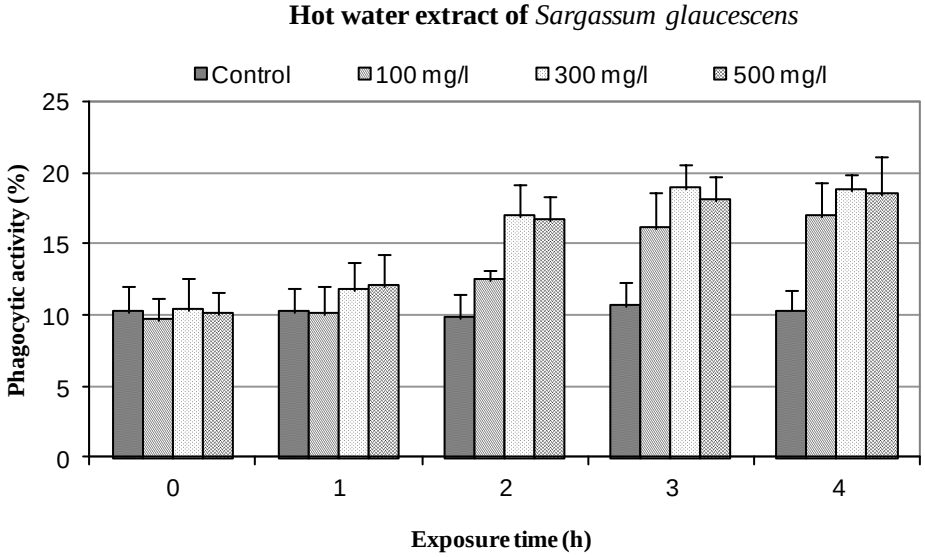

Figure 3: Mean (±SE) phagocytic activity (%) 0f *F. indicus* immersed in seawater containing hot-water extracts of *S. glaucescens* at 500, 300 and 100 mg/l, and the control shrimp.

Phagocytic activity

The phagocytosis percentage of control shrimps was within the range of 9.62±1.56 and 18.95±1.61 % (P>0.05) (Fig 3). The phagocytic activity of shrimp that were immersed in hot-water extracts of *S. glaucescens* at 100 mg/l was significantly higher than control shrimp after 3 and 4h, and at 500 mg/l and 300 mg/l was significantly higher than control shrimp after 2h.

Bactericidal activity

Bactericidal activity was significantly higher for the shrimp immersed in hot water extracts of *S. glaucescens* more than control (Fig 4A). Bactericidal activity increased from 8% in control shrimp to 15%, 23% and 22% for the shrimp immersed in 100, 300 and 500 mg/l, respectively.

Bacterial clearance efficiency

Clearance efficiency was significantly higher for the shrimp immersed in 100 mg/l hot water extracts of *S. glaucescens* more than 3h and immersed in 300 and 500 mg/l more than 2h (Fig 4B). Clearance efficiency increased by 43%, 53% and 60% for the shrimp immersed in 100, 300 and 500 mg/l, respectively, as compared to the control shrimp.

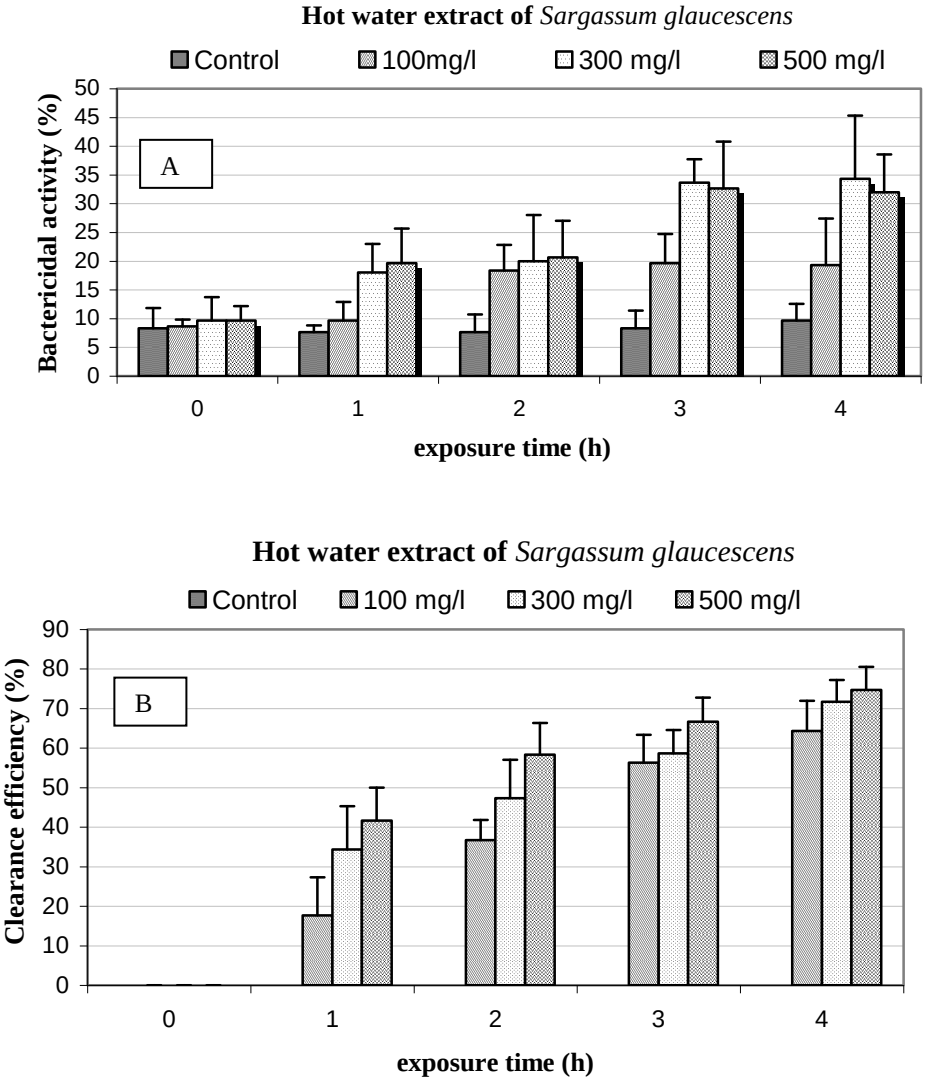

Figure 4: Mean (±SE) Bacterial clearance efficiency (%) (A) and Clearance efficiency (%) (B) 0f *F. indicus* immersed in seawater containing hot-water extracts of *S. glaucescens* at 500, 300 and 100 mg/l, and the control shrimp.

Discussion

Shrimp farming is a global industry that admeasures significantly to the economic development of many countries from tropical, subtropical and temperate areas. However, all producing countries have suffered drastic collapses due to the emergence of numerous pathogens and viral diseases since 1980 (Kautsky et al., 2000). Bacterial and Viral diseases have flamed within the past decade and enzootic pathogens causing serious mortalities in shrimp farms (Lightner et al., 1997). To prevent bacterial disease occurrence in larval production, zootechnical progress has been made (Robert and Gerard, 1999). Hitherto, a wide range of chemicals such as vitamins, disinfectants and antibiotics have been intensively used to treat water as preventive and curative agents (Barg and Lavilla-Pitogo, 1996). Administration of antibiotics has led to drug resistance in bacteria (Karunasagar et al., 1994) and can eke eventuate in environmental imbalances (Kautsky et al., 2000). Nowadays utilization of probiotic bacteria and immunostimulants as prophylactic methods for disease control in shrimp culture has been embraced.

Recent studies denoted that the extracts, containing a polysaccharide fraction, from several species of brown algae had an efficient ability to improve the immune responses or disease resistance of cultured aquatic animals (Chotigeat et al., 2004). The crude extracts of some algae were enunciated to impede only Gram positive bacteria e.g. *P. gymnospora* and *Dictyota dichotoma* extracts and the *Hypnea musciformis* extracts showed activity against *Salmonella typhosa* ParaA (Rao and Parekh, 1981). Crude extracts of *Sargassum muticum* can inhibit growth of a wide range of marine bacteria (Hellio et al., 2001). The specific fraction of the extract having antibacterial activity was not identified.

For crustaceans, some results emphasize on the importance of THC in pathogen and environmental stress resistance. Namely, Persson et al. (1987) reported in *Pacifastacus leniusculus* a relationship between THC and its resistance to *Aphanomyces astaci*. They demonstrated that a decline in THC of crayfish harboring *A. astaci* as a latent infection resulted in an acute infection and conduced to the death of the crayfish. Le Moullac et al. (1998) reported that *Penaeus styliriostris* with low THC resulting from hypoxia became more susceptible to infections with *Vibrio alginolyticus*. About environmental stress, it was observed that *Crangon crangon* exposed to the dredge spoils was found to evince a decrease in THC (Smith et al., 1995). *P. stylirostris* displayed a decline in THC after exposure to ammonia at 3 mg/l, (Le Moullac and Haffner, 2000).

In the current study, trend of changes in THC were similar to other studies but THC was counted less than similar studies perhaps because of the differences in some physical or chemical parameters such as salinity and temperature. The Bushehr province is in the north coast of the Persian Gulf and seawater salinity grows up to more than 40 ppt in August and September. The salinity was increased after sea water moved over to culture ponds and could have the role of a stressor

for shrimp and affect the shrimp physiology (Wang and Chen, 2006). Perchance this is one of the reasons for the decrease of THC in our study. The same results were obtained in the study on effects of *Padina boergesenii* by authors (data have not been published).

Plasma protein plays important roles in the immune system of crustaceans. It correlates with the infection of the pathogen (Vogan and Rowley, 2002; Song et al., 2003) and with environmental stress (Chen et al., 1992a, b); hence, it is a very serious immunologic parameter in the shrimp culture management because various immune molecules have been identified and purified in crustaceans such as lipopolysaccharide binding protein, β glucan binding protein and clotting protein (Sritunyalucksana and Soderhall, 2000). Two peptides were isolated with molecular masses of 73 and 75 kDa that had nonspecific antiviral properties and no cytotoxicity against host cells from *Penaeus monodon* hemocyanin (Zhang et al., 2004).

Hot-water extracts of several species of red and brown algae were found to increment the resistance of various fish and shrimp species against bacterial infections (Fujiki et al., 1992; Cheng et al., 2004, 2005). Oral administration of fucoidan extracted from *Sargassum polycystum* has been reported to decrease infection of the white spot disease in *P. monodon* (Chotigeat et al., 2004). Several polysaccharides were extracted from marine algae and challenged to boost non-specific immune system in teleosts and shrimp. For example, sodium alginate extracted from brown alga *M. pyrifera* provoked the migration of head kidney phagocytes to the peritoneal cavity after intraperitoneal injection. Laminaran extracted from *L. digitata* was observed to increase the activity of the ProPO system in *Farfantepenaeus californiensis* (Fujiki et al., 1994) and tiger shrimp (*P. monodon*) in an in vitro study (Sritunyalucksana et al., 1999). Injection and dietary administration of sodium alginate extracted from *M. pyrifera* has increased PO activity in *L. vannamei* (Cheng et al., 2004, 2005). Administration of laminaran extracted from brown alga *Laminaria digitata* in immersion route for 3h at 2 mg/ml provoked an increase in the release of superoxide anion in *L. vannamei* (Campa-Cordora et al.., 2002).

Phagocytosis can be affected by environmental parameters in invertebrates (Bayne, 1990). According to the results, the set of exchange in PA, BA and BCE was well correlated with the amount of THC and TPP. Thus viewpoints of other investigators on the importance of THC and TPP for evaluating the health situation were supported.

In the present study, the *F. indicus* received hot-water extracts of *S. glaucescens* through immersion rout which increased the THC, TPP, phagocytic activity, bactericidal activity and bacteria clearance efficiency. Therefore, hot-water extracts of *S. glaucescens* activate the non-specific immune system in both shrimps. The shrimps immersed in hot-water extracts of *S. glaucescens* at 100, 300 and 500 mg/l significantly ($p < 0.05$) had higher THC, TPP, phagocytic activity, bactericidal activity and bacteria clearance efficiency. Both the phagocytic activity

and clearance efficiency of *F. indicus* to *S.aureus* and *V. harveyi* increased significantly. Because of supertanker traffic in the Persian Gulf and bioaccumulation of pollutions and heavy metal ability of algae, some immunological index present minor response to studied hot water extract but proper results accrued and similar studies corroborate these results.

Because of supertanker traffic in Persian Gulf and bioaccumulation of pollutions and heavy metal ability of *P. boergesenii* like other algae (Sukhoon & Bhuguni 2001) and effects of heavy metal on haemocyte number in shrimp (Lorenzon, Francese, Smith & Ferrero 2001), some immunological index present minor response to evaluated immunostimulant efficacy of hot water extract, but proper results accrued and similar studies corroborate these results.

Acknowledgments

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References

Afsharnasab, M., S. Akbari, B. Tamjidi, F. Laloi and Soltan, M., 2006. Occurrence of white spot syndrome disease in farmed *Penaeus indicu* in Iran. Regional Aquaculture Information System.

Balasubramanian, G., Sarathi, M., Venkatesan, C., Thomas, J. and Sahul Hameed A. S., 2008. Studies on the immunomodulatory effect of extract of Cyanodon dactylon in shrimp, Penaeus monodon, and its efficacy to protect the shrimp from white spot syndrome virus (WSSV). *Fish & Shellfish Immunology*, 25, 820–828.

Barg, U. and Lavilla-Pitogo, C. R., 1996. The use of chemicals in aquaculture: a brief summary of two international expert meetings. FAO Aquaculture Newsletter, 14, 12– 13.

Bayne, CJ. 1990. Phagocytosis and non-self recognition in invertebrates. Phagocytosis appears to be an ancient line of defense. *Bioscienc*, 40, 723e31.

Bradford, M. M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing protein–dye binding. *Analysis Biochemistry*, 72, 248– 254.

Campa-Cordora, A. I., Hernandez-Saaveda, N. Y., De Philippis R. and Ascencio F., 2002. Generation of superoxide-anion and SOD activity in haemocytes and muscle of American white shrimp (*Litopenaeus vannamei*) as a response to b-glucan and sulphated polysaccharide. *Fish & Shellfish Immunology*, 12, 353-366.

Chang C. F., Chen H. Y., Su M. S. and Liao I. C., 2000. Immunomodulation by dietary β-1, 3-glucan in the brooders of the black tiger shrimp *Penaeus monodon*. *Fish & Shellfish Immunology*, 10, 505-514.

Chen, J. C. and Lin, C. Y., 1992a. Effect of ammonia on growth of Penaeus penicillatus juveniles. *Comparative Biochemistry Physiology*, 101C, 443–447.

Chen, J. C. and Lin, C. Y., 1992b. Effect of ammonia on growth and molting of *Penaeus mondon* juveniles. *Comparative Biochemistry Physiolology*, 101C, 449– 452.

Cheng, W., Liu, C. H., Kuo, C. M. and Chen, J. C., 2005. Dietary administration of sodium alginate enhances the immune ability of white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. *Fish & Shellfish Immunology*, 18, 1-12.

Cheng, W., Liu, C. H., Yeh, S. T. and Chen, J. C., 2004. The immune stimulatory effect of sodium alginate on the white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. *Fish & Shellfish Immunology*, 17, 41-51.

Chotigeat, W, Tongsupa, S, Supamataya, K and Phongdara, A., 2004. Effect of fucoidan on disease resistance of black tiger shrimp. *Aquaculture*, 233, 23-30.

Emadi H., Mooraki N., Matinfar A. and Negarestan H., 2010. Growth comparison between post-larvae from cultured and wild spawners of Indian white shrimp, Penaeus indicus, in commercial farms in north Persian Gulf, Bushehr, Iran. *Iranian Journal of Fisheries Sciences*, 9(**3**), 360-367.

Fujiki, K., Matsuyama, H., and Yano, T., 1992. Effect of hot-water extracts from marine algae on resistance of carp and yellow tail against bacterial infections. Science Bulletin, Faculty of Agriculture. *Kyushu University*, 47, 137-41.

Fujiki, K., Matsuyama, H. and Yano, T., 1994. Protective effect of sodium alginates against bacterial infection in common carp, *Cyprinus carpio* L. *Journal of Fish Diseases*, 17, 349-55.

Fujiki, K and Yano, T., 1997. Effects of sodium alginate on the non-specific defense system of the common carp (*Cyprinus carpio*). *Fish & Shellfish Immunology*, 7, 417-427.

Hellio, C., De La Broise, D., Dufosse, L., Le Gal, Y. and Bourgougnon, N., 2001. Inhibition of marine bacteria by extracts of macroalgae, potential use for environmentally friendly antifouling paints. *Marine Environment Research*, 52, 231– 247.

Houwen, B., 2000. Blood Film Preparation and Staining Procedures Laboratory Hematology 6,1–7 Carden Jennings Publishing Co., Ltd, Official Publication.

Jiang, G., Yu R. and Zhou, M., 2004. Modulatory effects of ammonia-N on the immune system of *Penaeus japonicus* to virulence of white spot syndrome virus. *Aquaculture*, 241, 61–75

Kakoolaki, S., Sharifpour, I., Soltani, M., Ebrahimzadeh Mousavi, H. A., Mirzargar, S. and Rostami, M., 2010. Selected morpho-chemical features of hemocytes in farmed shrimp, *Fenneropenaeus indicus* in Iran. *Iranian Journal of Fisheries Sciences*, 9(2), 219-232.

Karunasagar, I., Pai, R., Malathi, G. R. and Karunasagar, L., 1994. Mass mortality of *Penaeus monodon* larvae due to antibiotic resistant Vibrio harveyi infection. *Aquaculture*, 128, 203–209.

Kautsky, N., Ro¨nba¨ck, P., Tedengren, M. and Troell, M., 2000. Ecosystem perspectives on management of disease in shrimp pond farming. *Aquaculture*, 191, 145– 161.

Le Moullac, G. and Haffner, P., 2000. Environmental factors affecting immune responses in Crustacea. *Aquaculture*, 191, 121–131.

Le Moullac G., Soyez C., Saulnier D., Ansquer D., Avarre J.C. and Levy, P., 1998. Effect of hypoxic stress on the immune response and the resistance to vibriosis of the shrimp *Penaeus stylirostris*. *Fish and Shellfish Immunology*, 8, 621– 629.

Lightner, D. V., Redman, R. M., Poulos, B. T., Nunan, L. M., Mari, J. L. and Hasson K. W., 1997. Risk of spread of penaeid shrimp viruses in the Americas by international movement of live and frozen shrimp. *Epizootic*, 16, 140– 160.

Liu, C. H., Yeh, S. T., Cheng, S. Y. and Chen, J. C., 2004. The immune response of the white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio* infection in relation with the moult cycle. *Fish and Shellfish Immunology*, 16, 151-61.

Lorenzon, S., Francese, M., Smith, V. J., Ferrero, E. A., 2001. Heavy metals affect the circulating haemocyte number in the shrimp *Palaemon elegans*. Fish & Shellfish Immunology 11, 459–472.

Persson, M., Cerenius, L. and Soderhall, K., 1987. The influence of haemocytes number on the resistance of the freshwater crayfish, *Pacifatacus leniusculus* Dana, to the parasitic fungus *Aphanomices astaci*. *Journal of Fish Diseases,* 10, 471–477.

Rao, P. S. and Parekh, K. S., 1981. Antibacterial activity of Indian seaweed extracts. *Botany Marine*, 25, 577–582.

Robert, R. and Gerard A., 1999. Bivalve hatchery technology, the current situation for the pacific oyster *Crassostrea gigas* and the scallop *Pecten maximus* in France. *Aquatic Living Resource*, 12, 121–130.

Robertson, L., Bray, W., Leung-Truillo, J. and Lawrence, A., 1987. Practical molt staging of *Penaeus setiferus* and *Penaeus stylirostris*. *Journal of World Aquaculture Society*, 18, 180-5.

Sakai, M., 1999. Current research status of shrimp immunostimulants. *Aquaculture*, 172, 63-92.

Smith, V. J., Brown, J. H. and Hauton, C., 2003. Immunostimulation in crustaceans, does it really protect against infection. *Fish & Shellfish Immunology*, 15, 71-90.

Smith, V. J., Swindlehurst, R. J., Johnston, P. A. and Vethaak, A. D., 1995. Disturbance of host defense capability in the common shrimp, *Crangon crangon*, by exposure to harbor dredge spoils. *Aquatic Toxicology*, 32, 43–58.

Song, T. L. and Shiel, Y. H., 1995. Immunostimulation of shrimp *Penaeus monodon* Haemocytes to generate the microbicidal substances. *Aquaculture*, 98, 101-108

Song Y.L, Cheng W. and Wang C.H., 1993. Isolation and characterization of *Vibrio damsela* infectious for cultured shrimp in Taiwan. *Journal of Invertebrate Pathology*, 61, 24-31.

Song, Y. L. and Huang, C. C., 1999. Application of immunostimulants to prevent shrimp diseases. Recent advances in marine biotechnology. Immunobiology and pathology, Science Publishers, Inc. 173-88.

Song, Y. L., Yu, C. I., Lien, T. W. C., Huang, C. and Lin, M. N., 2003. Haemolymph parameters of Pacific white shrimp (*Litopenaeus vannamei*) infected with Taura syndrome virus. *Fish and Shellfish Immunology*, 14, 317–331.

Sritunyalucksana, K., Sithisarn, P., Withayachumnarnkul, B. and Flegel, T. W., 1999. Activation of phenoloxidase, agglutinin and antibacterial activity in haemolymph of the black tiger prawn, *Penaeus monodon*, by immunostimulants. *Fish & Shellfish Immunology*, 9, 21-30.

Sritunyalucksana, K. and Soderhall, K., 2000. The proPO and clotting system in crustaceans. *Aquaculture*, 191, 53–69.

Sukhoon, D. and Bahuguna, A., 2001. Evaluation of Padina boergesenii (Phaeophyceae) as a bioindicator of heavy metals: some preliminary results from Mauritius. South African journal of botany 67, 460-464.

Vargas-Albores, F., Gu zman, M. A. and Ochoa, J. L., 1993. An anticoagulant solution for haemolymp collection and prophenoloxidase studies of penaeid shrimp (*Penaeus californiensis*). *Comp. Biochemistry Physiology*, 106A, 299– 303.

Vogan C. L. and Rowley A. F., 2002. Effects of shell disease syndrome on the haemocytes and humoral defences of the edible crab, *Cancer pagurus*. *Aquaculture*, 205, 237– 252.

Wang F. I. and Chen J.C., 2006. Effect of salinity on the immune response of tiger shrimp Penaeus monodon and its susceptibility to Photobacterium damselae subsp. Damselae. *Fish and Shellfish Immunology*, 20, 676-81.

Weeks-Perkins B. A., Chansue N. and Wong-Verelle D., 1995. Assay of immune function in shrimp phagocytes, techniques used as indicators of pesticide exposure. In, Stolen, J.S., Fletcher, T.C., Smith, S.A., Zelikoff, J.T., Kaattari, S.L., Anderson, R.S., Soderhall, K. and Weeks-Perkins, B.A. (Eds.), *Techniques in Fish Immunology*, vol. 4. SOS Publications, Fair Haven, NJ, USA, 223– 231.

Yano, T., Matsuyama H. and Mangindaan, R. E. P. 1991. Polysaccharide-induced protection of carp, *Cyprinus carpio* L., against bacterial infection. *Journal of Fish Diseases*, 14, 577-82.

Yeh, S., Lee, C., and Chen, J., 2005. Administration of hot-water extract of brown seaweed *Sargassum duplicatum* via immersion and injection enhances the immune

resistance of white shrimp *Litopenaeus vannamei*. *Fish and Shellfish Immunology*, 20, 332-345.

Zhang X. B., Huang C. H. and Qin Q. W., 2004. Antiviral properties of hemocyanin isolated from shrimp *Penaeus monodon*. *Antivirus Research*, 61, 93–99.

تأثیر استفاده از عصاره آب گرم جلبک قهوه ای *Sargassum glaucescens* به روش غوطه وری بر پاسخهای ایمنی میگوی سفید هندی *(Fenneropenaeus indicus)*

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چکیده

توسعه پرورش میگو، علی رغم ضرورت جهانی آن، مشکلات اکولوژیک و پاتولوژیک بسیاری را برای کشورهای تولید کنندهٔ میگو ایجاد کرده است. سیستم ایمنیِ میگو، عنصری کلیدی در طراحی استراتژیهای کنترلی، جهت کنترل بیماریهای تهدید کننده این صنعت می باشد. در این مطالعه تعداد هموسیت کل (THC)، تعداد افتراقی هموسیت ها (DHC)، میزان پروتئین پلاسمای کل (TPP)، فعالیت فاگوسیتی، توان حذف باکتریایی و توانایی باکتری کُشی، در میگوهای سفید هندی با میانگین وزنی (۱/۲۰g ±۱۱/۳۲) پس از غوطه وری در آب دریا (شوری ppt ۳۹ و دما ($1^{\circ}C \pm 25$) حاوی ۱۰۰، ۳۰۰ و mg/l ۵۰۰ عصاره آب گرم جلبک قهوه ای *Sargassum glaucescens* پس از ۱، ۲، ۳ و ۴ ساعت غوطه وری اندازه گیری و تعیین گردید. این پارامترها در تیمار mg/l ۱۰۰ پس از ۳ ساعت و در تیمارهای ۳۰۰ و mg/l ۵۰۰ پس از ۱ ساعت غوطه وری افزایش معنی دار نشان داد. میگوهای سفید هندی پس از ۲ ساعت غوطه وری در غلظت های ۳۰۰ و mg/l ۵۰۰ افزایش مغنی داری را در فعالیت فاگوسیتی و توان خذف باکتریهای *.Vibrio spp* مشاهده گردید اما فعالیت باکتری کُشی در برخی از تیمارها پس از ۱ ساعت نیز افزایش معنی داری را نشان داد.

واژگان کلیدی: میگوی سفید هندی، سارگاسوم گلوسیسنس، تعداد هموسیت کل، تعداد افتراقی هموسیت ها، میزان پروتئین پلاسمای کل، فعالیت فاگوسیتی، توان حذف باکتریایی

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