SciMedCentral

Annals of Aquaculture and Research

Research Article

# Stimulatory Role of Low viscous Sodium Alginate Extracted from Turbinaria decurrens (Phaeophyta) on Immune Responses and Gene Expression in Litopenaeus vannamei

**Kokilam G, Vasuki S*, Deivasigamani B, and Babitha D**

*Department of Marine Biology, Annamalai University, India*

***Corresponding author**

Vasuki S, Department of Marine Biology, Annamalai University, CAS in Marine Biology, Faculty of Marine Sciences, Parangipettai, Tamil Nadu, India, Email: vasukipackiaraj04@yahoo.com

**Submitted:** 12 December 2016

**Accepted:** 30 December 2016

**Published:** 15 March 2017

**ISSN:** 2379-0881



**Keywords**

- Immunostimulants
- Sodium alginate
- Immune parameter
- *Litopenaeus vannamei*
- *Vibrio parahaemolyticus*
- Disease resistance

**Abstract**

This study assessed the stimulatory role of supplementation of low viscous sodium alginate extracted from *Turbinaria decurrens* (Phaeophyta) on the immune response of the white shrimp *Litopenaeus vannamei*. The total haemocytes count, phenoloxidase activity, respiratory burst and lysozyme activity of shrimp fed with diets containing sodium alginate was significantly higher than that of control (without sodium alginate). An increase in resistance against *V. parahaemolyticus* was observed in shrimps under laboratory conditions. An enhanced activity of βGBP, PE, proPO, cyt-SOD, PA-5 and SWDP mRNA transcriptions was observed in shrimps when fed with sodium alginate. In addition to that, expression fold of PE, pro PO and SWDP was higher with low viscous sodium alginate when compared to higher viscous sodium alginate. Our results suggest that low viscous sodium alginate supplementation plays a significant role in the immune responses and gene expression in *Litopenaeus vannamei*.

## INTRODUCTION

The commercial shrimp farming is largely based on culturing tiger shrimp (*Penaeus monodon*) kuruma shrimp (*Marsupenaeusjaponicus*) and white shrimp (*Litopenaeus vannamei*) and this has been predominantly affected by infectious pathogens, such as bacteria and viruses [1]. In general, crustaceans have not acquired immunity, as an alternative they have an innate immune system, which comprises of melanisation by activation of the prophenoloxidase activating system (pro PO system), a clotting process, phagocytosis, encapsulation of foreign material, antimicrobial action, and cell agglutination [2].

The immune stimulatory role of polysaccharides have been broadly studied and reviewed in fin fishes and shellfish [3]. The alginates extracted from brown seaweeds plays a role in enhancing the oxygen transference through the cellular membrane of fish lymphocytes and macrophages, so increasing the metabolic activity, resulting in improved disease resistance [4-10]. The hot-water extract of brown sea weed *Sargassum duplicatum* enhanced the immune resistance of white shrimp *Litopenaeus vannamei* against *V. alginolyticus* infections [11]. Sodium alginate has been reported to increase the resistance of *Cyprinuscarpio* against *Edwarsiellatarda* infection [12], and the non-specific defense system of *C. carpio* [6].

However, the immune genes transcription of shrimp consequent to immunostimulators administration is yet to be explained [13], has reported that the proPO system in haemocytes of the white shrimp *Litopenaeus vannamei* serves an important function in non-self-recognition and host immune reactions. There is a significant similarities in the primary structures of proPO, peroxinectin and clotting protein (CP) from shrimp and crayfish. Also, it has been demonstrated that the immune genes and immune defense are likely to be very similar and hence much knowledge can be learned from studies on crustaceans in general [14].

The present study was aimed at assessing the immune factors and immune gene expression in white shrimp *Litopenaeus vannamei,* after feeding the shrimps with diets containing low and high viscosity sodium alginate extracted from brown seaweed.

## MATERIALS AND METHODS

### Preparation of test diets

The sodium alginate was extracted from *Turbinaria decurrens* (brown alga) by the method described by [15,16]. The extract was mixed with commercially available artificial pellet feed. The four types of formulated diet are Type (1)-control (without alginate); Type (2)-standard (sodium alginate-Sigma product); Type (3)-1% and Type (4)-2 %) was prepared using the extract. For the present study the viscosity of alginate was taken into consideration for preparing the pellet feed. The viscosity of alginate obtained from *Turbinaria decurrens* was low (14 mPa-s) whereas the standard alginate (Sigma product) used was high (38 mPa-s). For coating, the feed pellets was mixed with the extract solution and incubated at room temperature for 7 days. After the absorption of the extract, it was coated with the binding gel to prevent dispersion of the extract in water. The feed was then dried and stored at room temperature until given to shrimp [17].

### Collection and maintenance of experimental shrimp

*Litopenaeus vannamai* (white shrimp) were collected from culture pond near Sirkali (Lat. 11°29" N and Long. 79°46" E), Tamil Nadu, south east coast of India. Shrimp length ranged from 7.3 to 9.0 cm and weight 8 to 9.5 g. Shrimps (n=150) were cultured in circular tank (150 L) and acclimated to the laboratory conditions for 15 days before the experiment. During acclimation period, the shrimps were fed twice daily with commercial pellet feed. In the tank, aeration was provided at regular interval and 50% of water was exchanged daily to maintain the water quality. During the culture periods, water temperature, 29.0±0.5°C; pH, 7.5±1.3 and salinity, 32±3.1% was maintained.

### Immune parameters of *L. vannamei*

**Haemolymph collection:** Haemolymph (100 µl) was collected from the ventral sinus of each shrimp into a 1 ml sterile syringe (25 gauge) and 0.9 ml of anticoagulant solution (0.45 M sodium chloride, 0.1 M glucose; 30 mM sodium citrate, 26 mM citric acid, 10 mM EDTA, pH 7.5 and an osmolality of 780 mOsm $kg^{-1}$). The remaining haemolymph mixture was used for subsequent tests.

**Total haemocytes count:** A drop of the anticoagulant-haemolymph mixture was placed on a haemocytometer to measure total haemocytes count (THC). The haemocytometer was observed under light microscope (Magnius MLX) and a count in all 4 squares was done using routine method. Haemocyte count=NxD/A x 10 x 103 cells $ml^{-1}$ (where, N=total number of cells counted; D = dilution of haemolymph; A = total area counted (in $mm^2$); 10 = factor to convert area to volume (µl), assuming a chamber of 0.1 mm depth; 103 = factor to convert count per µl to count per milliliter).

**Total phenoloxidase (PO) activity:** Total phenoloxidase activity was determined by using L-Dihydroxyphenylalanine (L-DOPA) [18]. Briefly, 100 µl of centrifuged haemolymph was mixed with 50 µl phosphate buffered saline (PBS) solution and 50 µl of enzyme inducer trypsin (Hi Media. 1 mg $ml^{-1}$) and incubated for 15 min at 25°C in 96-microliter plates (flat bottomed). In controls, trypsin and serum were replaced by PBS. After incubation, 100 µl of a L-DOPA solution (10 mg $1ml^{-1}$) was added to that mixture and incubated for 10 min at 25°C. Then the sample were read at 490 nm using a VERSA maxtunable microplate reader (Associates of Cape Cod Incorporated, East Falmouth, MA, USA) [19].

**Reduction of NBT by haemocytes:** To determine the amount of superoxide anion, the reduction of nitrobluetetrazolium (NBT) by haemocytes was measured. Haemolymph (100 µl) was placed in a microplate and incubated for 30 min at room measured. The supernatant was discarded and 50 µl of 0.3% NBT were added and incubated for 2 h at room temperature. The supernatant was again discarded, and the haemocytes were fixed with 200 µl of absolute ethanol. Haemocytes were washed twice with 200 µl 70% methanol and was allowed to dry. The formazan deposits generated were dissolved in 120 µl 2 M KOH and 140 µl dimethyl sulfoxide (DMSO) and optical density at 620 nm was recorded using a microliter plate reader [20].

**Lysozyme activity:** Lysozyme activity was measured by the modified method of Minagawa [21]. In this turbidometric assay, 0.03% lyophilized Micrococcus lysodeikticus in PBS was used as substrate. Ten microliters of haemolymph was added to 250 µl of bacterial suspension in duplicate wells of a microliter plate and the reduction in absorbance of 490 nm was determined after every minutes (1,2,3,4 and 5) of incubation at 22°C using a microliter plate reader. One unit of lysozyme activity was defined as a reduction in absorbance of 0.001 per min.

**Challenge with *Vibrio parahaemolyticus:*** In the present study, the bacterial pathogen *Vibrio parahaemolyticus* was used as a test organism. Lyophilized strain was used in this study. The bacterial strain was sub cultured and centrifuged at 10000g for 10 minutes at 4°C. The supernatant were discarded and the bacterial pellet was washed three times and resuspended in PBS at pH 7.4. The OD of the solution was adjusted to 0.5 at 456 nm which corresponded to 1x $10^7$ cells $ml^{-1}$. After sodium alginate treatment, shrimp were injected (50 µl) with *V. parahaemolyticus* (1x$10^7$ cells $ml^{-1}$) were observed for three weeks.

**Immune gene expression:** Haemolymph (0.50 ml) was collected from the ventral sinus cavity of each shrimp into a 1ml sterile syringe (with 25 gauge needle) containing 0.5 ml of precooled (4°C) anticoagulant solution (0.45 M sodium chloride, 0.1 M glucose, 30 mM sodium citrate, 26 mM citric acid, 10 mM EDTA, at pH 7.5 and with an osmolality of 780 mOsm $kg^{-1}$). The diluted haemolymph was centrifuged at 500 x g and 4°C for 20min, and the haemocyte pellet was washed once with cacodylate buffer (10 mM sodium cacodylate, 0.45 M sodium chloride, 20 mM calcium chloride; pH 7.0).The resulting haemocyte pellet was then used for the total RNA isolation.

**RNA isolation and RT-PCR of samples:** Total RNA was isolated from TRIZOL- (Sigma, India) according to the manufacturer's instructions. Briefly, the sample in TRIZOL was repeatedly pipetted to disrupt cells. The samples were incubated for 5 min at room temperature to permit complete dissociation

of nucleoprotein complexes, 0.25-ml portions of chloroform were added, and the samples were centrifuged at 12,000 x g for 15 min at 4°C. Upper aqueous phase was mixed with five milligrams of RNase-free glycogen and 0.5 ml of isopropyl alcohol were introduced to precipitate nucleic acids for 15 min at room temperature, and the pellets were washed with 75 % ethanol (in DEPC-treated water) (Invitrogen, U.S.A.). Pellets were resuspended in RNase free water, and DNase I (Invitrogen) treatment was performed according to the manufacturer's instructions. RT-PCR was performed in triplicate using Super Script TM two Step RT-PCR with platinum® Taq kit according to the manufacture's recommendations (Invitrogen, Carlsbad, CA, U.S.A.) [22].

For cDNA systhesis, complementary DNA was synthesized from 1 µg total RNA from each sample in 20 µL of reaction buffer (contained 50 mMTris-HCl, pH 8.3, 75 mMKCl and 3 mM $MgCl_2$) using Super Script II reverse transcriptase enzyme (Genetech, Rt-PCR mix- Germany) in a 20 µl volume reaction containing 10 mM dithiothreitol, 10 U RNase inhibitor (Promega, Madison, WI, USA), 1 mMdNTPs and 2.5 µM random hexamers. Each sample was incubated for 45 min at 45°C, followed by 1 min at 72°C in Agilent amplicon system (AGILENT Biosystems), the prepared cDNA was stored in -20°C for further use. The cDNA (1µl) was then amplified in 20 µl of reaction buffer for 40 cycles of denaturation (96°C for 30 s), annealing (56°C for 30s), and extension (72°C for 30 s) using primers. The immune genes studied were- βGBP (b-1,3-glucan-binding protein), proPO (prophenoloxidase), PE (peroxinectin), cyt-SOD (cytosolic SOD), PA-5 (penaeidin-5), SWDP (domain protein) and b-actin [23].

**Primer design:** The specific primer pairs were designed for each experimental gene's mRNA sequence using primer express software (Applied Biosystems, Foster City, CA, USA). The description is shown below. βGBP forward primer, 5'-CATGTCGAACTTCGCTTTCAGA-3' and reverse primer, 5'-CACCGCGTGGCATCTTG-3' (accession no.: AF368168); proPO forward primer, 5'-CGACTCCTGGATGCCATACAT-3' and reverse primer, 5'- CATCGCGAAGAG GAACTTTGT-3' (AF521948); PE forward GTTTCGCGGGCACATCTC-3' (AF188840); cyt-SOD forward primer, 5'- GGCTGGTACAGTCAGTCCTCAGA-3' and reverse primer, 5'-CCTCACCCAATTCAGCATTGA-3' (BI784454); PA-5 forward primer, 5'- GGCTAAG CCTGTGGCATGA-3' (AY326471); SWDP forward primer, 5'- GCCAGACGGATTGGGA TTG-3' and reverse primer, 5'-AGCCATTTATCAGGCAGCATATG-3' (BI784457); and b-actin forward primer, 5'- CACCACCGCTGAACGAGAA-3' and reverse primer, 5'- AAGG GCGACATAGCAAAGT TTTC-3' (AF100986).

RT-PCR was performed by monitoring the increase in fluorescence intensity of the SYBR Green dye with a Rotor-Gene 3000 Real-time PCR apparatus (Corbett Research) according to the manufacturer's instructions. All measurements were performed in triplicate. Real-time Rt-PCR data were represented as Ct values, where Ct was defined as the threshold cycle of PCR when amplified product was first detected. To minimize intra- and inter-assay variability caused by differences in PCR efficiency, the quantity of 5- tissue. The Ct or threshold value of the target sequence is directly proportional to the absolute concentration when compared with the threshold value for reference genes. The relative expression level of target gene were plotted as fold change compared to control and determined by the $2^{-\Delta\Delta ct}$ method [24], a relative quantification algorithm. The amount of the changed gene can be calculated by factor X with the formula: $X=2^{-\Delta\Delta ct}$. Where ΔΔct= (Ct of target) control- (Ct, of target x b-actin) sample.

## RESULTS AND DISCUSSION

In recent years, there considerable number of works emphasizing the potential benefits of immunostimulants to enhance the immunocompetence and disease resistance of cultured shrimp [25]. A 60-day feeding trial in beluga, *Husohuso* juvenile indicated that diet 4 g/kg alginic acid had the best response on the growth and immune system [26]. Similarly, brown-marbled grouper *Epinephelus fuscoguttatus* fed with diets containing sodium alginate and kappa carrageen an showed 100% survival for the fish that was fed with all diets after 14 weeks and no significant difference in growth was observed among the diets.

Earlier reports on *E. fuscoguttatus* which was fed with diet containing sodium alginate and k-carrageenan enhanced the innate immunity and increased the resistance from *V. alginolyticus* infection [27]. The present study supported the previous report where the supplementation of sodium alginate significantly influenced the immune response in *L. vannamei.*

The *Sargassum fusiforme* polysaccharide extract was assessed as a feed additive for juvenile shrimps of *Fenneropenaeus chinensis*, against vibriosis and immune activity. In general, oral administration of polysaccharide extract of 0.5 % and 1.0% for 14 days effectively improved vibriosis resistance and enhanced the immune activity of the shrimp [28]. Similar observations were recorded in the present study where diet with sodium alginate highly increased resistance against *V. parahaemolyticus* (Figure 1).

However, no significant differences in the total haemocyte count, SGCs, or GCs were observed in shrimp fed with sodium alginate diet [29] whereas [30] showed significantly increased SOD activity and decreased respiratory bursts. Also, in the present study total haemocytes count, phenoloxidase activity, respiratory burst and lysozyme activity of shrimp fed with diets containing sodium alginate was significantly higher than that of shrimp fed with control (without sodium alginate). Except total haemocytes count (Figure 2), the total phenoloxidase activity (Figure 3), NBT activity (Figure 4) and Lysozyme activity (Figure 5a & 5b) was found to be high in shrimps fed with low viscous sodium alginate diet. The diversity of PO activity and respiratory bursts induced by sodium alginate may be related to the species studied [29].

Like, in the present study [30] reported significantly increased βGBP and penaeidins gene expressions in shrimp fed with a diet containing sodium alginate. Also, the results clearly suggest that sodium alginate orally administered enhances the activation and activity of the proPO system and mechanism of phagocytosis which might result from βGBP and antibacterial peptide gene transcriptions in shrimp (Figure 6 and 7). [30] Indicated that *P. monodon* fed diets containing 1.0 and 2.0 g/kg sodium alginate had approximately 2.3 and 13.1-fold higher expressions of PE mRNA, respectively, than did shrimp fed the

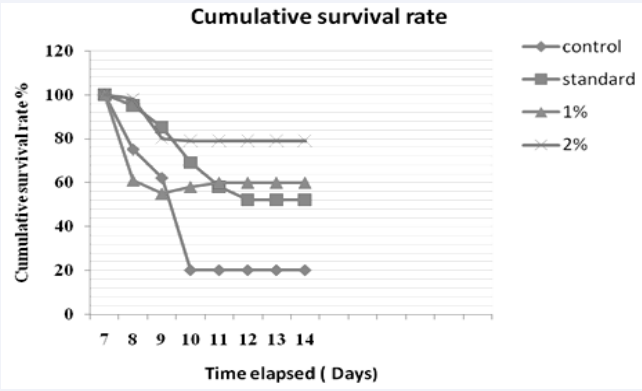


**Figure 1** Cumulative survival rate of *Litopenaeus vannamei* fed with different diets on 30th day.

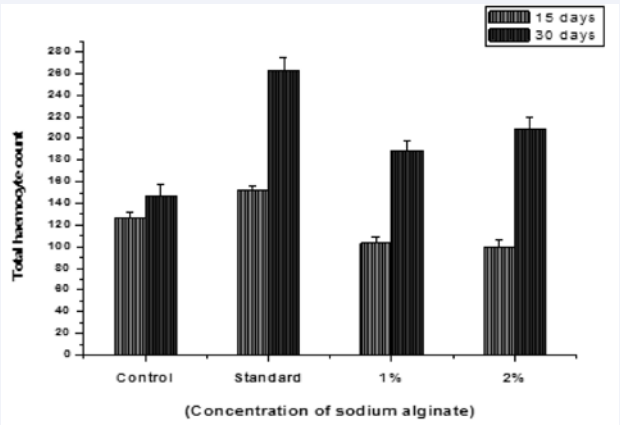


**Figure 2** The total haemocytes count of *Litopenaeus vannamei* fed with different diet on 30th day.

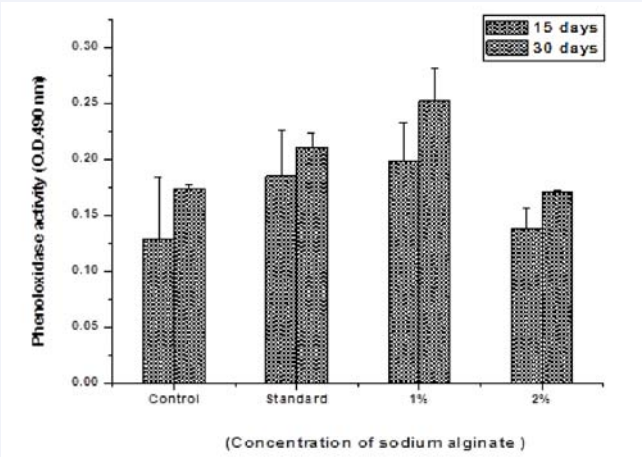


**Figure 3** Total Phenoloxidase activity of *Litopenaeus vannamei* fed with different diet on 30th day.

control diet. Like in the previous study, the present work also shows that shrimps fed with diet containing 1% and 2% sodium alginate had approximately 5.03 and 4.6-fold higher expressions of PE mRNA, respectively, than the control diet (Figure 8). These results suggest that sodium alginate enhances the cellular defence reaction by enhancing encapsulation and phagocytosis resulting from activation of the proPO system in *L. vannamei*. The expression fold of cyt-SOD (Figure 9) in *L. vannamei* was found to be high, whereas PA-5 showed more or less similar results (Figure 10) for both high and low viscous alginate diets. The expression of SWDP in *P. monodon* was remarkably increased with the sodium alginate containing diet [30] and more or less similar observations were made in *L. vannamei* (Figure 11). These facts suggest that sodium alginate enhances serine

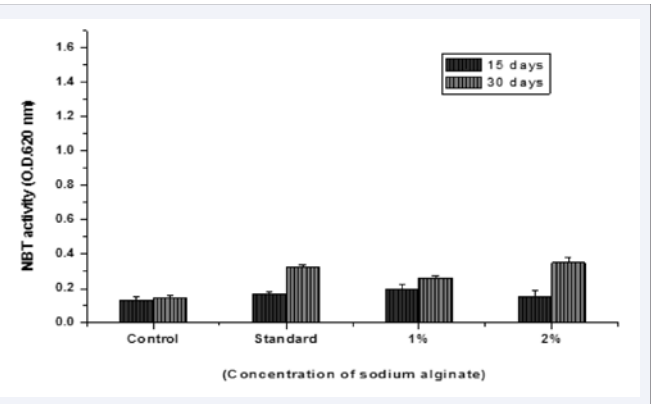


**Figure 4** NBT activity of *Litopenaeus vannamei* fed with different diet on 30th day.

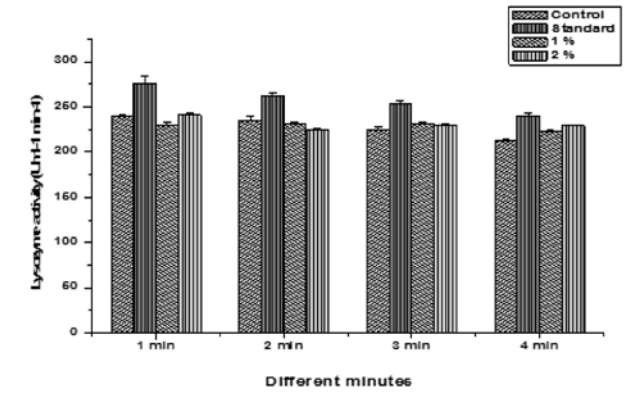


**Figure 5a** Lysozyme activity of *Litopenaeus vannamei* fed with different diets on 15th day.

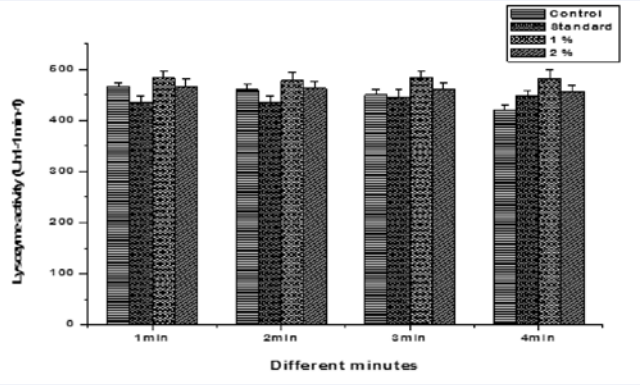


**Figure 5b** Lysozyme activity of *Litopenaeus vannamei* fed with different diets on 30th day.

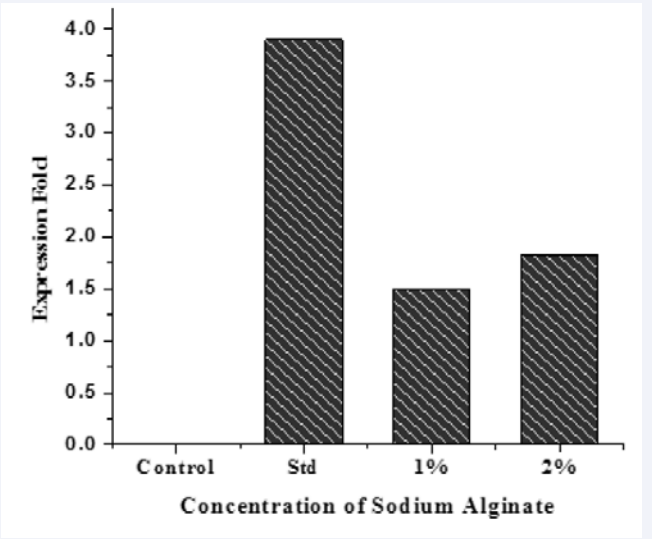


**Figure 6** Expression fold of βGBP in *Litopenaeus vannamei* fed with different diets on 30th day.

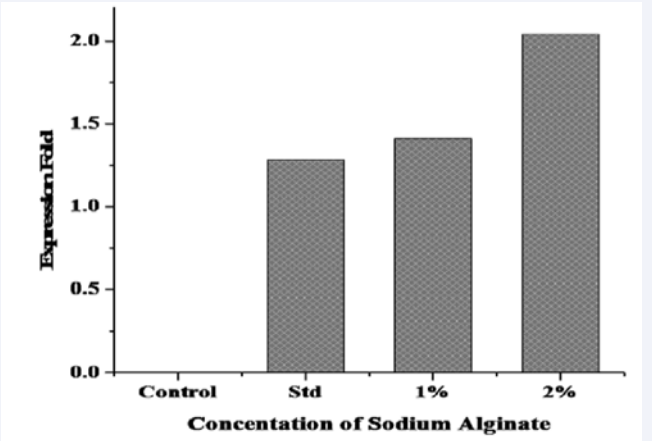


**Figure 7** Expression fold of proPO in *Litopenaeus vannamei* fed with different diets on 30th day.

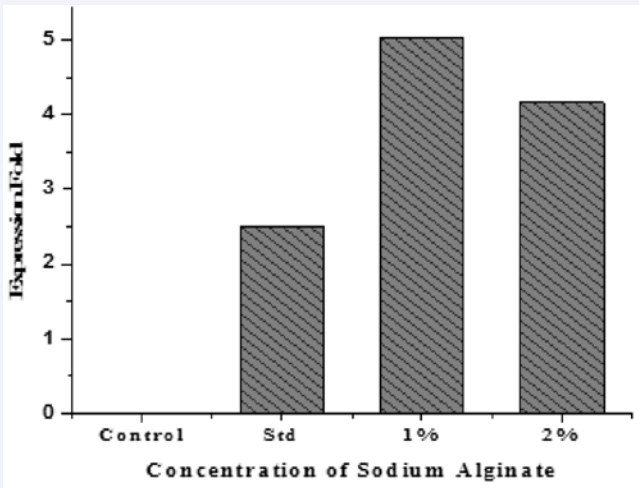


**Figure 8** Expression fold of PE in *Litopenaeus vannamei* fed with different diets on 30th day.

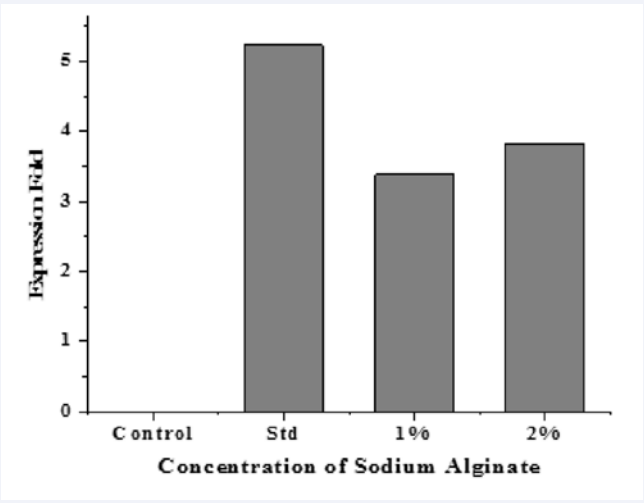


**Figure 9** Expression fold of cyt-SOD in *Litopenaeus vannamei* fed with different diets on 30th day.

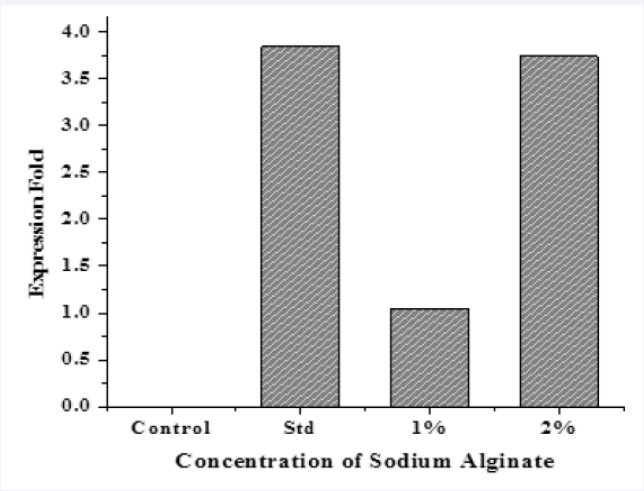


**Figure 10** Expression fold of PA-5 in *Litopenaeus vannamei* fed with different diets on 30th day.

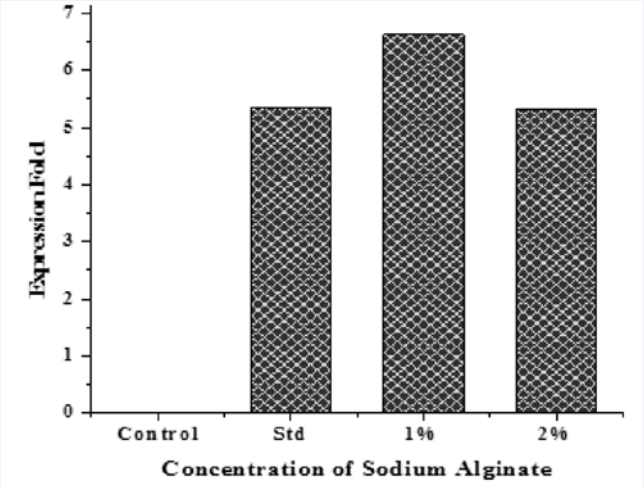


**Figure 11** Expression fold of SWDP in *Litopenaeus vannamei* fed with different diets on 30th day.

proteinase inhibitors and antibacterial activity to help regulate the immunity of shrimp.

Except few studies not much work has been done on immune gene expressions of shrimp. In most of the studies the trial period was longer whereas in the present study it was only for 30 days but at the same time the stimulatory role of sodium alginate was significant during this period.

## CONCLUSION

The expression fold of βGBP and cyt-SOD was higher with high viscous sodium alginate diet whereas for PE, proPO and SWDP it was with low viscous sodium alginate diet. The expression fold of PA-5 was more or less same in both high and low viscous sodium alginate diets. Hence, this study clearly shows the stimulatory role of low and high viscous sodium alginate on immune responses and gene expression in *Litopenaeus vannamei*.

## ACKNOWLEDGEMENTS

The authors are thankful to the Director, CAS in Marine Biology and to the authorities of Annamalai University for providing the necessary facilities to carry out this work.

## REFERENCES

1. Lo CF, Chang YS, Peng SE, Kou GH. Major viral diseases of Penaeus monodon in Taiwan. Journal of Fisheries Soc Taiwan. 2003; 30: 1-13.
2. Duvic B, Soderhall K. Purification and characterization of a b-1,3-glucanbinding protein from plasma of crayfish *Pacifastacus leniusculus*. J of Biological Chemistry. 1990; 265: 9327–9332.
3. Sakai M. Current research status of fish immunostimulant. Aquaculture. 1999; 172: 63-93.
4. Nussler AK, Thompson AW. Immunomodulatory agents in the laboratory and clinic. Parasitology. 1992; 105: 5-23.
5. Dalmo RA, Seljelid R. The immunomodulatory effect of LPS, laminaran and Sulphated laminaran ($(1,3)-D -glucan) on *Atlantic salmon, Salmo salar L*. macrophages *in vitro*. Journal of Fish Diseases. 1995; 18: 175-185.
6. Fujiki K, Yano T. Effects of sodium alginate on the non-specific defence system of the common carp (*Cyprinuscarpio L.*). Fish and Shellfish Immunology. 1997; 7; 417-427.
7. Miles DC, Polchana J, Lilley JH, Kanchanakhan S, Thompson KD, Adams A. Immuno stimulation of striped snake head Channastriata against epizootic ulcerative syndrome. Aquaculture. 2001; 195: 1-15.
8. Peddie S, Zou J, Secombes CJ. Immunostimulation in the rainbow trout (*Oncorhynchus mykiss*) following intraperitoneal administration of Ergosan. Veterinary Immunology and Immunopathology. 2002; 86: 101-113.
9. Vollstad D, Bogwald J, Gaserod O, Dalmo RA. Influence of high-M alginate on the growth and survival of Atlantic cod (*Gadusmorhua L.*) and spotted wolfish (*Anarhichas minor Olafsen*) fry. Fish and Shellfish Immunology. 2006; 20: 548-561.
10. Cheng AC, Tu CW, Chen YY, Nan FH, Chen JC. The immuno stimulatory Effects of sodium alginate and iota-carrageenan on orange-spotted grouper Epinephelus coicoides and its resistance against *Vibrio alginolyticus*. Fish and Shellfish Immunology. 2007; 22: 197-205.
11. Yeh ST, Lee CS, Chen JC. 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. 2006; 20: 332-345.
12. Fujiki K, Matsuyama H, Yano T. Protective effect of sodium alginates against bacterial infection in common carp, *Cyprinuscarpio L*. Journal of Fish Diseases. 1994; 17: 349–355.
13. Lai CY, Cheng W, Kuo CM. Molecular cloning and characterization of prophenoloxidase from haemocytes of the white shrimp, *Litopenaeus vannamei*. Fish and Shellfish Immunology. 2005; 18: 417-430.
14. Sritunyalucksana K, Soderhall K. The proPO and clotting system in crustacean. Aquaculture. 2000; 191: 53–69.
15. Suzuki N. Studies on the manufacture of algin from brown algae, Memoirs of the Graduate School of Fisheries Sciences Hokkaido University. 1955; 3: 93-158.
16. Ganesan M, Mairh OP PV. Effect of shelf life of brown algae *Sargassum wightii* (Fucales/Phaeophyta) and *Turbinaria conoides* (Fucales/Phaeophyta) on alginic acid yield. Indian Journal of Marine Sciences. 2001; 30: 108-110.
17. Balasubramanian G, Sarathi M, Venkatesan C, John Thomas AS, Sahul Hameed. Oral administration of antiviral plant extract of *Cynodondactylon* on a large scale production against White spot syndrome virus (WSSV) in *Penaeus monodon*. Aquaculture. 2008; 279: 2–5.
18. Perazzolo LM, Barracco MA. The prophenoloxidase activating system of the shrimp, *Penaeus paulensis* and associated factors. Developmental and Comparative Immunology. 1997; 21: 385-395.
19. Mexia-Salazar AL, Hernandez-Lopez J, Burgos-Hernadez A, Cortez-Rocha MO, Castro-Longoria R, Ezquerr-Brauer JM. Role of fumonisin B-1 on the immune system, histopathology and muscle proteins of white shrimp *Litopenaeus vannamei*. Food Chemistry. 2008; 110: 471-479.
20. Munoz M, Cedeno R, Rodriguez J, Van der Knaap WPW, Mialhe E, Bachere E. Measurement of reactive oxygen intermediate production in haemocyte of the penaeid shrimp, *Penaeus vannamei*. Aquaculture. 2000; 191: 89-107.
21. Maggioni DS, Andreatta ER, Hermes EM, Barracco MA. Evaluation of some Hemato-immunological parameters in female shrimp *Litopenaeus vannamei* submitted to unilateral eyestalk ablation in association with a diet supplemented with super doses of ascorbic acid as a form of immune stimulation. Aquaculture. 2004; 241: 501-515.
22. Grifantini R, Sebastian S, Frigimelica E, Draghi M, Bartolini E, Muzzi A, et al. Identification of iron-activated and dependent genes by transcriptome analysis of Neisseria meningitidis group B. Proceedings of the National Academy of Sciences, USA. 2003; 100: 9542–9547.
23. Sambrook J, Russel D. Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 3rd ed. 2001; 1: 14–15.
24. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-Time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001; 25: 402–408.
25. Traifalgar RFN, Corre VL, Serrano AE. Efficacy of dietary immunostimulants to enhance the immunological responses and vibriosis resistance of juvenile Penaeus monodon. Journal of Fisheries and Aquatic Science. 2013; 8: 340-354.
26. Ahmadifar E, AzariTakami GH, Sudagar M. Growth Performance, Survival and Immunostimulation, of Beluga (Husohuso) Juvenile Following Dietary Administration of Alginic Acid (Ergosan). Pakistan Journal of Nutrition. 2009; 8: 227-232.
27. Cheng AC, Chen YY, Chen JC. Dietary administration of sodium

alginate and kappa-carrageen an enhances the innate immune response of brown-marbled grouper *Epinephelus fuscoguttatus* and its resistance against *Vibrio alginolyticus*. Veterinary Immunology and Immunopathology. 2008; 121: 206-215.

28. Huang X, Zhou H, Zhang H. The effect of Sargassum fusiforme polysaccharide extracts on vibriosis resistance and immune activity of the shrimp, Fenneropenaeus chinensis. Fish and Shellfish Immunology. 2006; 20: 750-757.

29. Cheng W, Liu CH, Kuo CM, Chen JC. Dietary administration of sodium alginate enhances the immune ability of white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. Fish and Shellfish Immunology. 2005; 18: 1–12.

30. Liu CH, Yeh SP, Kuo CM, Cheng W, Chou CH. The effect of sodium alginate on the immune response of tiger shrimp via dietary administration: Activity and gene transcription. Fish and Shellfish Immunology. 2006; 21: 442-452.

**Cite this article**

*Kokilam G, Vasuki S, Deivasigamani B, Babitha D (2017) Stimulatory Role of Low viscous Sodium Alginate Extracted from Turbinaria decurrens (Phaeophyta) on Immune Responses and Gene Expression in Litopenaeus vannamei. Ann Aquac Res 4(1): 1030.*