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RESEARCH ARTICLE

EEFICACY OF PROBIOTIC SUPPLEMENTED DIETS ON GROWTH POTENTIALS AND IMMUNE RESPONSES IN WHITE SHRIMP *LITOPENAEUS VANNAMEI*

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ABSTRACT

Monitoring of growth performance studies were conducted with pacific white shrimp *Litopenaeus vannamei* after fed with three experimental diets i.e. commercial brand CP feed, feed formulated with plant based ingredients and feed formulated with animal based ingredients along with and without probiotics including soil, feed and water probiotics for a period of 120 days. Growth patterns of *L. vannamei* recorded highest growth potentials with probiotic supplemented commercial brand CP feed, followed by probiotic supplemented feed formulated with animal based ingredients and lastly feed formulated with plant based ingredients. The growth patterns were recorded for every 30 days and the culture operation was continued up to 120 days. Growth parameters including Weight gain (g), Specific growth rate (%), Average daily growth rates, Production rates, Feed conversion ratios were recorded in all the control and Experimental ponds. Haemolymph immune parameters including Total Haemocyte count, Hyaline cells, Semi-Granular cells, Granular Cells, Prophenol oxidase and Total protein contents were different experimental feeds with and without probiotic supplementation. The results obtained clearly demonstrate that due to supplementation of probiotics during culture operation reduced the pathogen load and lead to reduction in the breakdown of diseases and finally increased the productivity in terms of yield. Therefore, the probiotic supplementation will pave way for the triggering of growth rates significantly thereby increasing the productivity.

INTRODUCTION

Shrimp farming is one of the most profitable aquaculture sectors in the world, and plays an important role in the socio-economic development of the country, provides protenacious food for the poor people. The Aquaculture practices has expected to account progressively for the insufficient aquatic food supply that would occur for the population increase until 2030, and it is the fastest growing food production sector in the world increasingly with an average rate of 9.22% over the past 30 years (FAO, 2014), which makes aqua industry one of the promising industries to meet the future food demands effectively. The pacific white shrimp *Litopenaeus vannamei* has become the main crustacean species produced through culture, with production exceeding that of tiger shrimp *Penaeus monodon* since 2003 (FAO, 2007). *L. vannamei* is the primary penaeid shrimps currently being cultured in all over the world including Asia, especially in India. The Aqua industry faced several setbacks due to revenging bacterial and viral diseases. The use of Antibiotics in production has been criticized due to the potential development of Antibiotic resistant Bacteria, the presence of Antibiotic residues in seafood, destruction of the microbial population in the aquatic environmental suppression of the animal's immune system (Ng *et al.,* 2009). Therefore, the immune ability of shrimp and their susceptibility of shrimp and their susceptibility tom pathogens are of primary concern when they are subjected to environmental stressors. Penaeid shrimp like other invertebrates rely on an innate immune system for protection against pathogens (Anderson, 1992). Once microorganisms or other foreign particles invade the haemocoel of the host, they encounter a complex system of innate defence mechanisms involving cellular and humoral responses (Takahashi *et al.,* 1995). By keeping the above information of the present investigation aimed to probe into the growth aspects of *L. vannamei* during culture operation and also studying the responses of certain immune related parameters in prawns after fed with different commercial feeds, plant based or Animal based ingredient formulated feeds.

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MATERIALS AND METHODS

The present investigation was carried out in shrimp culture farms located in and around Ramayapatnam (Latitude 15^{o} 02^{1} 55^{11} N; Longitude 80^{o} 02^{1} 50^{11} E) Prakasam Dist., Andhra Pradesh, South India during September – December, 2016. The entire farm has a covered area of 15 ha and three ponds were selected for the present study. The ponds selected in the

present investigation are having the average size of 1.5 ± 0.1 ha and are rectangular in shape with clay loamy soil suitable for semi intensive type of culture operation. The water source selected for culture operation was drawn from Buckingham canal, which was very close proximity with the culture farm, with the help of Motors. During the culture operation the Hydrological conditions including Salinity (12 ± 1 ppt), pH (7.5 ± 0.2), Transparency (35-55 cm), Dissolved oxygen (3.8 ± 0.2 ppm) and Temperature (30 ± 2° C) were maintained. In all the experimental ponds selected were maintained a water column of 1.5 mts depth. Initially all the selected ponds were allowed to dry and splinter to increase the capacity of oxidation of Hydrogen supplied and to eliminate the fish eggs, crab larvae and other unwanted predators. The pond bottom was scrapped 3 to 5 cm by using a tractor blade to avoid top soil. Then the pond bottom was ploughed horizontally and vertically to a depth of 30 cm to remove the obnoxious gases, increase fertility. The soil pH was recorded in the ponds with the help of cone type pH meter. For increasing the availability of nutrients, required amount of lime was applied to neutralize the acid soil, condition of the soil based on the average pH level of the pond. *Litopenaeus vannamei* of 0.62 ± 0.03 g were obtained from local hatcheries and were stocked in different experimental ponds at the rate of 25 pcs/M^2 with a stocking rate of 2,00,000 for each pond. Six ponds were selected in the present investigation. Pond-1/2 were fed with commercial brand feed i.e. CP brand feed along with probiotics. Pond-3/4 were fed with an experimental diet formulated with plant materials as principle ingredients along with probiotics. Pond-5/6 were fed with experimental diet formulated with Animal materials as principle ingredients along with probiotics. In the present investigation soil, water and feed probiotics selected Biomax, Probio Aqua and Feed Act, respectively. Biomax was broadcasted at the rate of 4-5 kg/ha (1 M water depth), Probio Aqua 4 lit/ha (1 M water depth) and Feed Act 10 g/kg feed were applied by following the manufacturer's instructions. The culture period was continued for a period of 120-121 days. The composition of all the experimental diets for mulated were presented in Table 1.

All the groups of shrimps were fed their respective diet at a rate of 8-10% of body weight each day. This daily ration was divided into two equal feedings at 6:00 AM and 6:00 PM. Intermittent aeration was done in all the experimental ponds depending upon the need and necessity. Growth performance studies were conducted for 120 days with every fortnight i.e. 15 days and the observations were recorded. Growth parameters including Average Body weights, Average Daily growth rates, Specific growth rates, Feed Conversion Ratio were monitored and tabulated. The growth parameters can be calculated by adopting the following formulas.

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total Amount of Feed Broad Casted (kgs)}}{\text{Total Biomass of Prawns (kgs)}} \times 100$$

$$\text{Survival Rate (\%)} = \frac{\text{Total Number of Live Animals}}{\text{Total Number of Seed Stocked}} \times 100$$

$$\text{Weight gain} = \text{Weight of the Animal (G) at the end of culture period} - \text{Weight of the Animal (G) at the time of Stocking}$$

$$\text{Average Daily growth rates} = \frac{\text{Weight of the prawn (G) at the end of the culture period} - \text{Weight of the Prawn (G) at the time of Stocking}}{\text{Total number of days of culture operation}}$$

$$\text{Specific growth rates} = \frac{\text{Log weight of the prawn at the end of the culture period} - \text{Log weight of the prawn at the time of Stocking}}{\text{Total number of days of culture operation}} \times 100$$

$$\text{SGR:} \quad \frac{(\text{Log } W_2 - \text{Log } W_1)}{T} \times 100$$

Where

W_1 : weight of the prawn at the start of the culture operation
W_2 : weight of the prawn at the end of the culture operation
T : Total number of days of culture operation

Haemoly mph extraction and Haemocyte identification:

Haemolymph was withdrawn from the ventral sinus of each shrimp with a 1 ml sterile syringe (26 guage) containing of anticoagulant solution (0.45 M Nacl, 0.1 M glucose, 10 mM EDTA, 30 mM Sodium citrate, 26 mM citric acid, pH 7.3), a modification from the solution described by Soderhall and Smith (1983). A drop of the anticoagulante-haemolymph mixture (100 µL) was placed on a Haemocytometer to measure Hyaline cell, Granular Cell and Semi granular cell and the Total haemocyte count and differential haemocyte count were determined using an inverted phase-contrast microscope (Olympus). The reminder of the haemolymph mixture was used for subsequent tests. Phenol oxidase (PO) activity was measuredSpectrophotometrically by recording the formation of Dopachrome produced from L -3,4dihydroxy phenylalanine (L-DOPA) is previously by described by Liu *et al.* (2004) and Hemandez *et al.* (1996). Optical density at 490 nm of the shrimps PO activity was measured using Spectrophotometer and expressed as Dopachrome formation per 50 µL of Haemolymph. The total haemolymph protein was also estimated by the method Lowry *et al* (1951).

RESULTS AND DISCUSSION

In the present investigation, Growth performance studies of *Litopenaeus vannamei* was conducted. Six ponds were selected in the present investigation and all the ponds were stocked with 2,00,000 seed of *L.vannamei* obtained from local hatcheries. Pond-1 was treated as control and the prawns were fed with CP Brand commercial feed without probiotics, whereas Pond-2 was broadcasted with CP Brand commercial feed and probiotics including soil, water and feed probiotics were also used during culture operation. Similarly, Pond-3 and Pond-4 were broadcasted with feed formulated based on plant ingredients without probiotics and with probiotics, respectively. Pond-5 and Pond-6 were broadcasted with feed formulated based on Animal ingredients without probiotics and with probiotics, respectively. The culture operation was carried out for a period of 120-121 days. Results pertaining to growth patterns were monitored for every 15 days and presented in Table 2 and 3. From the results obtained for growth patterns of *L. vannamei*, clearly indicates that among the three diets selected in the present investigation commercial diet i.e. CP Brand feed induced highest and best growth potentials compared to feeds formulated with plant or Animal based ingredients. Moreover, the growth patterns of prawns also recorded highest with the usage of probiotics in all the culture ponds, irrespective of the three feeds used compared to without probiotics.

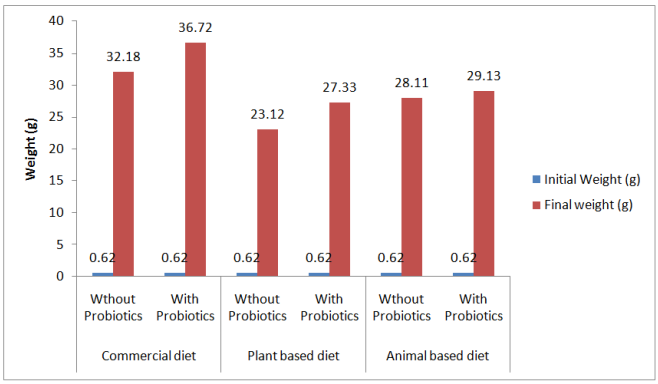

Figure 1. Average Body weights of *L.vannamei* after fed with Experimental diets during culture operation

So, the use of soil, water and feed probiotics in the present investigation demonstrates that use of probiotics induces best growth potentials compared to without probiotics use in culture ponds. All the parameters related to growth performance includes Weight gain, Specific growth rate, Average daily growth rate, Production rates, Feed consumed and Feed conversion ratios were observed to be more significant with CP Brand commercial feed compared to feeds formulated with plant or Animal based ingredients either with or without the use of probiotics. Similar kind of observations were also reported in *L. vannamei* when fed with different feeds in the presence of water, feed and soil probiotics (Pratap Reddy *et al.,* 2016) and also in freshwater prawn *Macrobrachium rosenbergii* (Rangappa, 2011; Saad *et al.,* 2009).

Table 1. Composition of Basal Diets (as Percentage dry weight)

Ingredients	Plant based Diet	Animal based Diet	
Choline Chloride	0.50	0.50	
Ca $(H_2Po_4)_2$	0.42	0.42	
Vitamin Premix	0.50	0.50	
Mineral Premix	1.00	1.00	
Cholesterol	1.00	1.00	
Cellulose	0.50	0.50	
Plant materials			
Groundnut cake	15.00	3.92	
Soybean	25.00	Fish meal 21.08	
Corn Gluten	15.00	Shrimp meal 15.00	
Peanut meal	10.00	Squilla meal 15.00	
Rice bran	10.00	Squid meal 15.00	
Soy oil	2.00	Snail meal 15.00	
wheat flour	14.58	Poultry meal 15.00	
Starch	4.50		
Proximate composition			
Crude Protein	37.33 ± 1.54	38.15 ± 1.28	36.74 ± 1.12
Total Lipid	5.38 ± 0.21	6.74 ± 0.24	7.43 ± 0.28
Fibre	5.12 ± 0.21	4.83 ± 0.18	4.88 ± 0.21
Moisture	11.75 ± 1.32	18.32 ± 1.28	18.94 ± 1.33
Ash	14.08 ± 1.22	17.33 ± 1.28	16.85 ± 1.24

Table 2. Growth performance of white shrimp *L.vannamei* during culture operation after fed with different experimental diets

Days of culture	Commercial diet				Plant based diet				Animal based diet			
	without Probiotics		With Probiotics		without Probiotics		With Probiotics		without Probiotics		With Probiotics	
	IBW (g)	GI (g)	IBW (g)	GI (g)	IBW (g)	GI (g)	IBW (g)	GI (g)	IBW (g)	GI (g)	IBW (g)	GI (g)
0	0.62 ± 0.03		0.62 ± 0.03		0.62 ± 0.03		0.62 ± 0.03		0.62 ± 0.03		0.62 ± 0.03	
30	2.49 ± 0.13 (+302)	1.87	2.68 ± 0.14 (+332)	2.06	2.03 ± 0.12 (+227)	1.41	2.28 ± 0.14 (+227)	1.66	2.28 ± 0.13 (+268)	1.66	2.39 ± 0.14 (+285)	1.77
60	9.74 ± 0.28 (+291)	7.25	11.99 ± 0.29 (+347)	9.31	8.15 ± 0.27 (+302)	6.12	10.13 ± 0.24 (+344)	4.01	8.74 ± 0.29 (+283)	6.48	10.77 ± 0.23 (+351)	8.38
90	22.49 ± 0.38 (+131)	12.75	27.74 ± 0.39 (+131)	15.75	18.14 ± 0.22 (+123)	9.99	22.18 ± 0.32 (+119)	12.19	19.77 ± 0.29 (+126)	11.03	25.12 ± 0.31 (+133)	14.35
120	32.18 ± 0.41 (+43)	9.69	36.72 ± 0.44 (+32)	8.98	23.12 ± 0.29 (+27)	4.98	27.33 ± 0.34 (+23)	5.15	28.11 ± 0.33 (+42)	8.34	29.13 ± 0.32 (+16)	4.01

All values are Mean ± SD of Six individual observations. Parenthesis in brackets are percent change over Respective control.
IBW: Initial Body Weight GI: Growth Increment

Table 3. Growth parameters of white shrimp *L.vannamei* during culture operation after fed with different experimental diets

Parameter	Commercial diet		Plant based diet		Animal based diet	
	Wthout Probiotics	With Probiotics	Wth out Probiotics	With Probiotics	Wthout Probiotics	With Probiotics
Initial Weight (g)	0.62 ± 0.03	0.62 ± 0.03	0.62 ± 0.03	0.62 ± 0.03	0.62 ± 0.03	0.62 ± 0.03
Final weight (g)	32.18 ± 0.41	36.72 ± 0.44	23.12 ± 0.29	27.33 ± 0.34	28.11 ± 0.33	29.13 ± 0.32
Weight gain (g)	31.56	36.08	22.5	26.71	27.49	28.51
Weight gain (%)	5090	5819	3629	4308	4434	4598
SGR	1.429	1.471	1.309	1.370	1.380	1.393
ADGR	0.263	0.301	0.188	0.223	0.229	0.238
Survival (%)	92	92	92	91	92	92
Yield (Kgs)	5921	6756	4254	4974	5172	5246
Feed consumed (Kgs)	15098	15810	12251	13579	13861	14217
FCR	2.55	2.34	2.88	2.73	2.68	2.71

SGR: Specific Growth Rate ADGR: Average Daily Growth Rate
FCR: Feed Conversion Ratio

Table 4. Total Hemocyte count (THC), Hyaline cells (HC), Semi Granular cells (SGC), Granular cells (GC), Prophenoloxidase (PO) and Total

Hemolymph Protein (THLp) in *L. vannamei* after fed with different Experimental diets after 60 days of culture operation

Parameter	Fed with Commercial Diet		Fed with Plant Based Diet		Fed with Animal Based Diet	
	Without Probiotics	With Probiotics	Without Probiotics	With Probiotics	Without Probiotics	With Probiotics
THC (x 10^5 cells/ml)	22.75+2.14	34.32+3.15	14.49+1.35	22.14+2.12	19.13+1.42	29.75+2.42
	PDC	(+50.86)	PDC	(+52.80)	PDC	(+55.51)
HC (x 10^5 cells/ml)	11.32+0.74	15.14+0.92	6.02+0.28	8.28+0.78	8.14+0.29	12.45+0.88
	PDC	(+33.75)	PDC	(+36.88)	PDC	12.45+0.88
SGC (x 10^5 cells/ml)	5.37+021	8.05+0.38	3.11+0.12	4.13+0.22	5.13+0.29	7.46+0.34
	PDC	(+49.91)	PDC	(+32.80)	PDC	(+45.52)
GC (x 10^5 cells/ml)	9.77+0.68	14.72+0.88	6.82+0.61	9.73+0.75	9.44+0.43	13.41+0.82
	PDC	(+50.67)	PDC	(+42.67)	PDC	(+42.05)
PO (units/mg	21.38+0.59	38.44+0.79	10.39+0.24	15.74+0.38	20.13+0.54	29.39+0.48
Protein/min)	PDC	(+79.79)	PDC	(+51.49)	PDC	(+46.00)
TH_LP (mgs/lit)	29.93+1.04	48.74+1.95	20.15+0.84	29.78+0.85	27.72+0.78 PDC	41.35+1.08
	PDC	(+62.85)	PDC	(+47.79)		(+49.17)

All values are Mean + SD of six individual observations. All values are statistically significant at P<0.001 from their respective control.
PDC : Percent deviation over controls

The growth patterns observed to be more prominent between 30-60 days, 60-90 days and 90-120 days in all the three feed broad casted ponds either with probiotics or without probiotics and this has been revealed through the Average daily growth rates, Specific growth rates etc., obtained in all the ponds during culture operations. In all ponds, the survival rates of prawns were relatively very high ranging around 91-92% indicating that the culture environment is highly ideal for the culture operation and subsequently for the growth of the prawns. The production rates were also significant and ranges between 4254 kgs to 6756 kgs in all the ponds and moreover the probiotics used ponds produced highest yields compared to other ponds without probiotics use. The FCR and feed consumption rates also showed to be best with CP Brand commercial diet i.e. 2.55 compared to other experimental diets selected in the present investigation. In the present investigation, the highest final weights recorded are 36.72 g in Pond-2, with commercial diet CP Brand feed broadcasted with probiotics and 32.18 g in Pond-1 with commercial diet CP Brand feed broadcasted without probiotics. In between the feeds formulated with Animal or Plant ingredients, the feed formulated with Animal ingredients fared significantly better in inducing growth potentials *in L. vannamei* compared to feed formulated with plant ingredients. The results obtained clearly demonstrate that the feed formulated with Animal ingredients might be meeting the requirements of either EAA or Protein sources to prawns compared to feeds formulated with plant ingredients. Similar kind of observations were also observed in *L. vannamei* when feed with feeds of plant and Animal origin (Pratap Reddy *et al.,* 2016; Bhavani, 2015). The results obtained for growth rates clearly demonstrate that use of probiotics during the culture operation clearly enhances the production rates and therefore the probiotics are playing a positive role in enhancing the growth and growth related indices in *L. vannamei*. The prawns fed with commercial diet and diets formulated with plant or Animal ingredients showed considerable increase in all the parameters associated with immune system of prawns. Total Haemocyte count (THC), Hyaline cells (HC), Semi Granular cells (SGC), Granular cells (GC), Prophenoloxidase (PO) were founded to be significantly elevated in the haemolymph of *L. vannamei* after fed with three experimental diets. The results obtained in the present study clearly demonstrated that, a promising immune response stimulation of *L. vannamei* with fed with feeds along with probiotics. Shrimps have three types of circulating haemocytes: Hyaline cells, Semi granular cells and Large granular cells (Sung *et al.,* 1996), which are important for their association with the recognition and removal of foreign materials. Shrimp immune system is non-specific, and associated with shrimp haemocyte's pattern recognition, phagocytosis, relative oxygen species formation, prophenoloxidase activating system, encapsulation, nodule formation and release of antimicrobial peptides and lysozymes (Takahashi *et al.,* 1995; Jiravanichpaisal *et al.,* 2006; Hemandez-Lopez *et al.,* 1996). With elevated level of Haemolymph THC, there is an increased number of immune cells to combat infectious sources and subsequently, reduces mortality. Among all haemocyte types in shrimps, hyaline cells are the smallest shape with the highest nucleus to cytoplasm ratio. Because of the presence of three types of haemocytes has its niche of function, the varying proportions of the three cell types at a given time in dictate a particular immune function in progress. Due to feeding the probiotic containing diets, the total numbers of all three haemocytes were significantly higher compared to prawn fed with feeds but not with probiotics.

Among the three types of haemocytes, Semi granular cells are considered to be most sensitive and reactive (Cerenius *et al.,* 2010) and act as primary immune activator of the white shrimp with their high levels of granules, enzymes and proteins. Therefore, an increase in the proportions of semi granular cells can effectively enhance immune response in white shrimps. THC and DHC showed a significant increase in the haemolymph after fed with experimental feeds with probiotics, resulting in an increased immune response to infections. Haemocytes are associated with the pro-phenoloxidase (PO) system involved in encapsulation and mechanisation and which functions as a non self-recognition system (Smith and Soderhall, 1983; Johanson and Soderhall, 1989). The protein concentrations of haemolymph also shown to be increased significantly in prawns fed with experimental diets along with probiotics. The present investigation may be concluded that the growth potentials were prominently triggered by feeding with CP brand commercial feed along with probiotics supplementation, compared to prawns fed with plant or Animal based diets with probiotic supplements. Among the two experimental diets, the feed formulated with Animal based ingredients induced relatively more growth rates compared to the diet formulated with plant based ingredients. The probiotic supplementation in the culture operation of *L. vannamei* inducing the maximum growth potentials and there by the

productivity and also reducing the emergence of diseases in the culture operation. Therefore the probiotic supplementation in the *L.vannamei* culture operation is recommended.

REFERENCES

Anderson, DP, 1992. Immunostimulants, Adjuvants and vaccine carriers in fish: Applications to Aquaculture. *Ann. Rev. fish. Diseases.* 2:281-307.

Bhavani, M. 2015. Studies on the Determination of nutritional requirements for freshwater prawn *Macrobrachium rosenbergii*. Ph.D. Thesis. S.V.University, Tirupati.

Cerenius, L., Soderhall. K. 2011. Coagulation in invertebrates. J. Innate. *Immunol.* 3:3-8.

FAO. 2014. Food and Agriculture Organization of the United Nations. The status of world Fisheries and Aquaculture. Rome.

FAO. 2007. Food and Agriculture Organization of the United Nations. The status of world Fisheries and Aquaculture. Rome.

Hamandez-Lopez, J., Gollas-Galvan, T. and Vargas-Albores F. 1996. Activation of the prophenol oxidase system of the Brown shrimp *Penaeus californiensis*. Comp. *Biochem. Physiol*., 113:61-66.

Jiravanichipaisal, P., Lee, BL., and Soderhall. K. 2006.Cell-mediated immunity in Arthropods: Hematopoiesis, Coagulation, mechanization and opsonisation. *Immunology*, 211:213-236.

Johansson, MW. and Soderhall. K. 1989.Cellular immunity in crustacean and the pro PO system. Parasitol. *Today*. 5:171-176.

Liu, CH., JC Chen. 2004. Effect of ammonia on the immune response of white shimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus*. Fish. *Shellfish Immunol.,* 16:321-334.

Lowry, OH., Rosenbrough, NJ., AL Farr and RJ Randell. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.,* 193:265-276.

Ng, SC., Hart, AL., MA Kamm, AJ, Stagg. and CC, Knight. 2009. Mechanism of action of probiotics: Recent advances. *Inflamm. Bow. Dise*. 15:300-310.

Pratap Reddy. C, K. Hareesh, Y. Suneetha, M.S. Reddy. 2016. Efficacy of animal and plant feed ingredients in the formulation of feeds for induction of growth potentials in penaeid shrimp *Litopenaeus vannamei*. I*nt. J. Fish. Aquatic. Stu*. 4:7-14.

Rangappa A. 2011. Studies on the monitoring of growth patterns of giant freshwater prawn *Macrobachium rosenbergii.* Ph.D. Thesis. S.V.University, Tirupati.

Saad, SA., MHabashy, M. KK Sharshar. 2009. Growth response of the freshwater prawn *Macrobarchium rosenbergii* to diets having different levels of biogen. *World. App. Sci. J.* 6:550-556.

Soderhall, K., Smith, VJ. 1983. Induction of degradation and lysis of haemocytes in the freshwater crayfish *Astacus astacus* by components of the prophenoloxidase activating system *in vitro*. *Cell Tissue. Res.,* 233:295-303.

Sung, HH., YL, Yang., YL, Song. 1996. Enhancement of microbial activity in the tiger shrimp *Penaeus monodon* via immune stimulation. *J. Crustacean Biol*., 16:278-284.

Smith, VJ., Soderhall. K. 1983. Induction of degranulation and lysis of haemocytes in the freshwater crayfish *Astacus astacus* by components of the Prophenoloxidase activating system in vitro. *Cell Tissue Res.* 233: 295-303.

Takahashi, Y., Itami, T. and Kondo, M. 1995. Immuno defense system of crustacean. *Fish. Pathol.,* 30:141-150.

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