Int. J. Applied Bioresearch **ISSN 2250 - 2033 (Print); e -ISSN: 2250 - 2041 (Online)**

Immunostimulant responses on Methanolic leaves extract on *Syzygium caryophyllatum* (L.) supplemented with basal diets fed on *Vibrio parahaemolyticus* infected *Macrobrchium idae* (Heller, 1862)

Muthiah MARIDASS and Ganapathy RAJU

Department of Zoology, Pioneer Kumaraswamy College, (Affiliated to Manonmaniam Sundaranar University), Nagercoil,Tamil nadu-629003, India.
Curresponding Author Email maridassugcpdf@yahoo.co.in

Received: 08 January 2019 / Accepted: 11 February 2019/ Published Online:15 March 2019
http://www.gtrpcompany.com/ijab.htm

Citation: Maridass M, Raju G. Immunostimulant responses on Methanolic leaves extract on *Syzygium caryophyllatum* (L.) supplemented with basal diets fed on *Vibrio parahaemolyticus* infected *Macrobrchium idae* (Heller, 1862).Int.J.Appl. Bioresearch,2019;38:1-6.

Abstract

The aim of the present study was growth performance and histopathological analysis of freshwater prawns of *Macrobrchium idae* (Heller, 1862) fed with supplemented diet on the different concentration of methanolic leaves extract of *Syzygium caryophyllatum* (L.) for 4 weeks. The experimental study groups were 0% (Control), 1,2,3 and 4% concentrations of methanolic leaves extract of *S. caryophyllatum* (L.) supplemented with control diet. The methanolic leaves extract of *S. caryophyllatum* supplemented with basal diet fed with normal and *Vibrio* infected *M. idae* for 28 days observed by growth parameters, histological analysis of hepatopancreas. The results were observed by excellent growth on weight gain (%), the survival rate (%) and FCR rate (%) of *M. idea* for 28 days. 4% of extract *S. caryophyllatum* (L.) leaves diet was observed by 100% of survival rate. Control fed with *Vibrio parahaemolyticus* infected prawn *M. idae* was observed 100 % of mortality within one week. The control fed with *M. idae* was histologically observed by hepatopancreas is blind tubules, which is consisting of four type of cells. The E-cells at the summits of the tubules develop into R-cells (for resorption of nutrients), F-cells (for production of digestive enzymes) and B-cells (function unknown). Experimental diets fed with *M. idae* were observed by hepatopancreas was appeared to be healthy structure. The disease infected prawn *M. idae* fed with control diet was observed by unhealthy hepatopancreas conditions including slough hepatopancreas tubules cells, degeneration of tubules lumen, enlarged of the hepatopancreas nuclei cell and lack of B, F and R epithelial cells in the hepatopancreas tubules. The conclusion of the study shows that hepatopancreas conditions can be the indicator to determine the conditions of the prawn health status fed with all experimental diets. The conclusion of the present study may be acted as alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, phenolics, steroids and essential oils present in this plant. The plant extract of *Syzygium caryophyllatum* (L.) leaves was established in the potential use of additive for diets on freshwater prawn of *M.idae*.

Keywords: Freshwater, *Macrobrchium idea* (Heller, 1862), medicinal plants, bacteria, feeds, growth, pathology
.

1 INTRODUCTION

Global aquaculture production has been steadily increasing over the last decade. Aquaculture industries of Asian countries like Taiwan, Indonesia, Thailand and India have been emerged as global leaders in prawn production. Culture of prawn industry affected in many diseases in aquaculture industry. Disease and production problems vary during the different phases of shrimp culture. One of the most important criteria for a successful culture operation is insured to maintain good health of the shrimp. The environment can have a significant impact on prawn health,

growth and production. Success of culture depend on totally maintaining in the good health condition in shrimp with proper functions of various organs. The hepatopancreas a digestive gland or midgut gland is an organ of the digestive tract of arthropods (Shrimp) and mollusks. It provides the functions in mammals are provided separately by the liver and pancreas. Hepatopancreas is a gland that ends in ducts that open into the stomach.

Syzygium caryophyllatum L. is belongs to the family Myrtaceae. It is a tree native to Sri Lanka and south India, where it grows in the mid altitudes of the Ghats regions of Kerala, Karnataka and Tamil Nadu. *Syzygium caryophyllatum* L. is one of the species that has been categorized as endangered tree species under the international nature for conservation of nature (IUCN) red list of threatened species. It is known as Wild black plum. In Tamil it is known as Kattu naval. The fruit is edible. Previously, studied on antimicrobial, antioxidant, anticancer and antihyperglycemic activities of the leaf extract were reported [1-2]. In the present study was observed by immunostumilatory activity of *Syzygium caryophyllatum* L. leaves extract supplemented with basal diet fed on *Vibrio parahaemolyticus* infected *Macrobrchium idea* (Heller, 1862).

2 MATERIALS AND METHODS

2. 1 Diet Preparation

Prawns feeds were prepared for control diet and experimental diets in table-1. The proximal composition of basal and experimental diets. Feeds were given at the rate of 10% of body weight and formulated in table-1.

2.2 Preparation of plant extracts

The powdered plant materials were extracted with successively in methanol for 4hr in Soxhlet apparatus. Solvents were evaporated under reduced pressure and stored at °C for use.

Table -1: Proximal composition of basal and experimental diets.

Macromolecules	Diet-1 Control diet	Diet-2 with extract 2%	Diet-3 with extract 3%	Diet-4 with extract 4%
Protein	38-40%	38-40%	38-40%	38-40%
Carbohydrate	25-35%	25-35%	25-35%	25-35%
Lipid	3-7%	3-7%	3-7%	3-7%
Cholesterol	0.5-0.6%	0.5-0.6%	0.5-0.6%	0.5-0.6%
Ash	4% 5-6%	4% 5-6%	4% 5-6%	4% 5-6%

Percentage of macromolecule without extract

2.3 Experimental animals

The experimental prawns of *M. idae* (2±1g) were selected and acclimated to laboratory condition for 2 weeks before starting the experiments. The prawns *M.idae* were fed with twice per day at the rate of 5% of their body weight. Unconsumed food and excreta were removed every morning before feeding with replacement of 15% freshwater. During the experiment the mean physico-chemical parameters of water were as follows: water temperature 27°±1.0°C, pH 7.2 ±1, dissolved oxygen 8.1±0.3 mg/l, hardness as $CaCO_3$ 91±0.5 mg/l, NO_3-N 0.006±0.003 mg/l and NH_3 0.021±0.004 mg/l. The prawns were fed with the basal/experimental diet for 4 weeks. Growth performance studies were conducted continuously with every 7 days break and observations were recorded. Growth parameters including Average weights, Average growth rate (GR), Specific growth rate (SGR), Feed conversion ratio (FCR), Percentage of survival rate were monitored and tabulated.

2.4 Disease Challenge studies

Experimental setup (2.2) Sensitivity of rearing larval prawns to 50 strains was examined by the disease challenge test applying immersion method [27]. Healthy larvae and postlarvae of *M. idae* used for this study. Before and after the challenge, water and prawn samples were processed for bacteriological examination. In addition, bacteria isolated from experimental prawns were identified to confirm their identity with test strains. Pathogenicity tests demonstrated that several isolated bacteria *Vibrio parahaemolyticus* were pathogenic to larval prawns at concentration of 10^5 - 10^7 cell/ml. Survival rate was observed by 28 days of post challenge. The bacterial infected group, normal group of prawns of *M. idae* were fed with basal/experimental diet for 4 weeks. Growth performance studies were conducted continuously with every 7 days break and observations were recorded. Growth parameters including weight gain, growth rate (GR), Specific growth rate (SGR), Feed conversion ratio (FCR), Percentage of survival rate were monitored and tabulated.

2.5 Histopathology analysis

Normal and infected and treated prawn diseected in hepatopancreas were firstly fixed in the FAA solution for two days. The HP samples were placed in 70% alcohol before they were transferred into the tissue processor. The procedure for tissue processing were done as followed: Alcohol 70% for 1 h continued by alcohol 90%, alcohol 95%, alcohol 100%, (xylene I, xylene II, xylene III) for clearing and wax (2h)

continued with another wax (2h) for impregnation. After that the sample was embedded, cut and sectioned. Sectioned samples were dried on hot plate for 2 days. The samples were then stained using the hematoxylin and eosin procedure according to the procedure as followed: Xylene I (5 min), continued by xylene II (5 min), 100% alcohol I (5 min), 100% alcohol II (5 min), 95% alcohol (2 min), 70% alcohol (2 min), running tap water (2 min), hematoxylin (10 min), running tap water (2 min), acetone acid (3 dip), running tap water (2 min), 2% potassium acetate (3 min), running tap water (2 min), eosin (5 min), 95% alcohol (5 min), 95% alcohol (5 min), xylene I (5 min), xylene II (5 min) and finished with dpx mounting. The slide samples were then analyzed using microscope hanse lab. The infected and uninfected HP tissue was identified under the microscope and labeled for further data analysis.

2.5 Statistical analysis

The results are presented as mean values ± standard error. The data of different treatments, obtained on every thirty days, were submitted to one-way analysis of variance (ANOVA) with Tukey-Kramer Multiple Comparisons Test for all groups and Student's t test unpaired for comparison between two groups. Differences were considered significant at 5%. Statistical analyses were performed by GraphPad InStat 3.01.

3 RESULTS AND DISCUSSION

In the present study, different concentrations of *S. caryophyllatum* (L.) leaves extract supplemented with basal diet had increased the survival rate of *M. idea* compared than the control seen in table-2. The growth performance of *M. idea* was observed that 4% methanolic extract of *S. caryophyllatum* (L.) leaves best growth performances recorded for seen in the table-2.These were found to be significantly higher in the groups of prawn fed with 4% *S. caryophyllatum* (L.). Control diet was observed that showed lesser values compared to the experimental groups. The nutrition is regarded as a key factor controlling of survival and growth of crustacean species [20]. Enhanced nutrient intake directly correlates with the weight gain was observed in the present study. Control diet fed with *M. idae* was observed that hepatopancreas affected in changes of bacterial infection. 4% of experimental diets *S. caryophyllatum* fed with *M. idae* was observed by good condition of hepatopancreas and it was directly related to the growth performance and body health.

Table-2: Growth parameters of *Syzygium caryophyllatum* (L.) leaves extract with basal diet on different concentrations

Parameters	Control Diet	Experimental diet (1)	Experimental diet (2)	Experimental diet (3)	Experimental diet (4)
Survival rate (%)	70	90	90	90	100
Wait gain (%)	74.32±1.04	82.98±1.98	89.46±1.14	92.84±1.22	96.84±1.90
SCR (%)	1.24±1.12	1.08±1.98	0.96±1.34	0.78±1.56	0.64±1.11
FCR (%)	2.45±1.68	3.46±1.23	3.87±1.31	4.12±1.54	4.56±1.12

Table-3: Immunomodulatory activity of Growth responses of *Syzygium caryophyllatum* (L.) leaves extract with basal diet on different concentrations fed with bacterial infected M. idae

Parameters	Control Diet	Bacterial Infected control	Experimental diet (1)	Experimental diet (2)	Experimental diet (3)	Experimental diet (4)
Survival rate (%)	60	30	40	70	90	100
Wait gain (%)	34.12±0.14	12.43±0.14	56.23±0.31	60.11±1.12	60.12±1.45	96.84±1.34
SCR (%)	1.24±1.12	-	2.08±1.94	1.96±1.45	1.78±1.01	1.64±2.11
FCR (%)	1.45±1.68	-	2.46±1.26	2.87±1.11	2.12±1.54	3.56±1.45

The immunostimulant activity of *S. caryophyllatum* (L.) have been studied to basal diet incorporated with different percentage of extract of *S. caryophyllatum* (L.) fed with *V. parahaemolyticus* infected prawns *M.idae* observed for 28 days. Experimental diets of *S. caryophyllatum* (L.) fed with *M. idae* were observed by stimulate in the immune system enhanced by survival rate and growth performance (Table-3). Previously studies, *Emblica officinalis* fruits and leaves contain active constituent of vitamin C, which is a potent immunostimulant and antioxidant activities reported in several authors [3,15-17]. The ascorbic acid present in this fruit is conjugated to gallic acid and reducing sugars, forming a tannoid complex, which is more stable in nature and enhances the bioavailability of ascorbic acid [18]. Similarly, another constituent, Guduchi (*Tinospora cordifolia*), is well known to augment phagocytic cell functions and enhance protection against infections in animals and human beings. The other constituents, Aswagandha (*Withania somnifera*) and Tulsi (*Ocimum sanctum*) are also well known for their immunomodulatory activities [18]. The present study was observed by enhancing effects may be reflected on the immune parameters in treated prawns of *M.idae*.

The control fed with *M. idae* were observed by the destruction of hepatopancreas tissue. The hepatopancreas tissue were observed by *Vibrio parahaemolyticus* affected and the healthy prawns of *M.idae*. The present study showed the degeneration of the E, F, R epithelial tissue in HP and the collapse of the epithelial tubules in some of the prawn of *M.idae*. Lightner *et al*. (2012) reported that both species of *P. monodon* and *P. vannamei* affected shrimp, showed that the EMS trace limited to the hepatopancreas and that was described as lack of mitotic activity in E cells of hepatopancreas, dysfunction of central hepatopancreas B, F and R cells, massive sloughing of central HP tubule epithelial cells and massive intertubular hemocytic aggregation followed by secondary bacterial infections[13].

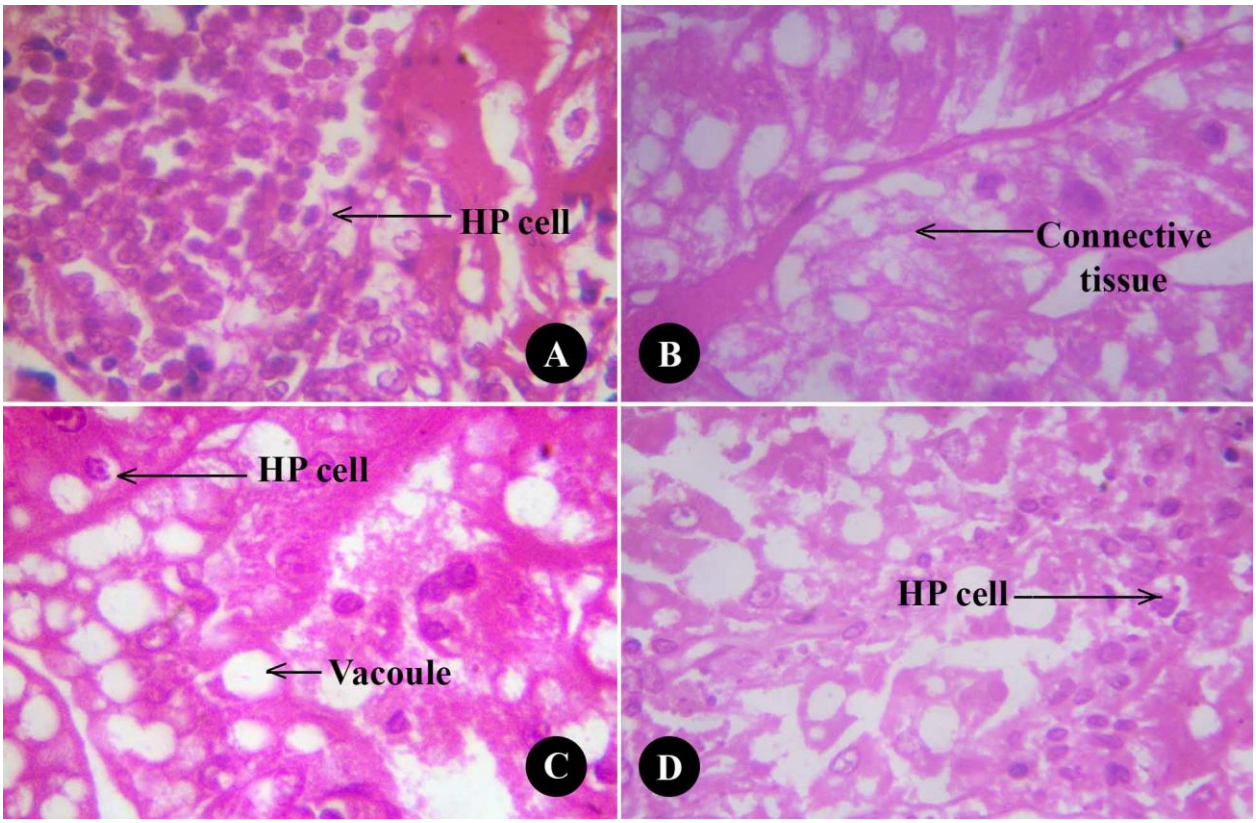

Fig.1 Histological section of Hepatopancreas of *M.idae*

The present study was different concentration of *S. caryophyllatum* (L.) extracts incorporated with basal diets fed with *M. idae* observed for 28 days and histologically section with 6µm of hepatopancreas (Fig.1). *M. idae* fed with control diet was observed by hepatopancreas showed in large compact ducts and blind ending tubules. Each tubule consists of single layer of epithelial cells enclosed a lumen. Previously studies examined in the haemocyte and muscle cells are found in the connective tissue around the tubules. According to Bhavan and Geraldine, (2000) reported that hepatopancreas is a very sensitive organ and liable to injury by pesticides and other water pollutants. Infected hepatopancreas was appeared poorly vacuolated indicating low lipid and glycogen reserves [14]. Jiravanichpaisal *et al*., (1994) observed that systemic vibriosis typically results in the formation of septic haemocytic nodules in hepatopancreas [4]. Previous studies, crustacean species of haemocytes was exhibited in degranulation *in vitro* when exposed to bacteria. While, granules in haemocytes from a variety of healthy decapod crustaceans are known to contain lysosomal enzymes, prophenoloxidase and antibacterial compounds. The haemocytes associated with the basal lamina of diseased shrimp *Sicyonia ingentis* were placed to light pathogens passing into the body through the mid gut. The lesion observed in the hepatopancreas of diseased prawns are likely to be structural manifestation of disruptions in the absorptive, storage and secretory functions of the hepatopancreas [12]. The destruction of hepatopancreas tissue observed maybe due to the disease affected the healthy prawn *M. idae*. The present study showed the degeneration of the E, F, R epithelial tissue in hepatopancreas and the collapse of the epithelial tubules. Earlier studies, Lightner et al. (2012) reported on both species of *P. monodon* and *P. vannamei* affected shrimp, showed that the EMS trace limited to the hepatopancreas and that was described as lack of mitotic activity in E cells of hepatopancreas, dysfunction of central hepatopancreas B, F and R cells, massive sloughing of central hepatopancreas tubule epithelial cells and massive intertubular hemocytic aggregation followed by secondary bacterial infections. Similar results were also obtained by Prachumwat et al. (2012) who identified by dysfunction of the tubule epithelial cells that progress from proximal to distal ends of

hepatopancreas tubules. This degenerative pathology strongly suggests a toxic etiology, but anecdotal information suggests that disease spread patterns may be consistent with an infectious agent [19]. Lightner (2013), the cause of EMS was thought to be a microbial infection with the *Vibrio parahaemolyticus* as the causative agent. The name of this disease was then changed to AHPND (Acute Hepatopancreatic Necrosis Disease) [13]. Lightner (2013) has identified the EMS pathogen as a unique strain of a relatively common bacteria *V. parahaemolyticus*, which had been infected by a virus known as a phage, caused it to release a potent toxin. A similar phenomenon occurs in the human disease cholera, where a phage makes the *Vibrio cholerae* bacterium capable of producing a toxin that causing cholera's life-threatening diarrhea [13]. Lightner et al. (2014) also observed acute degeneration of the hepatopancreas by initially decrease of R, B and F cell followed by marked reduction of mitotic activity in E cells, dysfunction of R, B and F cells and prominent karyomegally (enlarged nuclei) and sloughing into the hepatopancreas tubule lumen [13]. The conclusion of the present work observed that 4% of methanolic leaves extract of *S. caryophyllatum* supplemented with basal diet fed with *Vibrio parahaemolyticus* infected *M. idea* was observed by best immunostimulatory activities.

4 REFERENCES

1. Annadurai G, Masilla BR, Jothiramshekar S, Palanisami E, Puthiyapurayil S, Parida AK. Antimicrobial, antioxidant, anticancer activities of *Syzygium caryophyllatum* (L.) Alston. Int J Green Pharm 2012;6:285-8.
2. Savitha Rabeque C, Padmavathy S. Hypoglycaemic Effect of *Syzygium Caryophyllatum* (L.) Alston on Alloxan induced diabetic Albino Mice. Asian J Pharm Clin Res, 2013;6(l4): 203-205.
3. Anderson DP. Immunostimulants, adjuvants, and vaccine carriers in fish: applications to aquaculture, Ann Rev Fish Dis, 1992; 2: 281.
4. Jiravanichpaisal P, Miyazaki T, Limsuwan C. Histopathology, biochemistry and pathogenicity of *Vibrio harveyi* infecting black tiger prawn *Penaeus mondon*. J. Aquat. Ann. Health, 1994;6: 27-35.
5. Soderhall K, Smith VJ, Johansson MW. Exocytosis and uptake of bacteria by isolated haemocyte populations of two crustaceans: Evidence for cellular co-operation in the defense reactions of arthropods. Cell Tissue Res., 1986;2: 43-49.
6. Kalia V, Chaudhari S, Gujar GT. Changes in haemolymph constituents of American bollworm, *Helicoverpa armigera* (Hubner), infected with nucleaopolyhedrovirus. J. Exp. Biol, 2001; 39: 1123-1129.
7. Jussila J, Jaco J, Tsvetnenke E, Dunstan B, Evan LH. Total and differential haemocyte counts in Western rock lobster (*Panulirus gygnus* George) under post-harvest stress. Mar. Freshwater Res., 2004;48: 863-868.
8. Sharshar KM. Changes in haemolymph and biochemical constituents of cultured freshwater prawn *Macrobrachium rosenbergii* (Decapoda: Crustacea) infected with *Vibrio* sp. J. Union Arab Biol, 2004;21: 91-107.
9. Khoo L, Robinette DW, Noga EJ. Callinectin, an antibacterial peptide from blue crab, *Callinectes sapidus*, hemocytes. Mar. Biotechnol, 1999; 1: 44-51.
10. Destoumieux D, Munoz M, Cosseau C, Rodriguez J, Bulet P, Comp M, Bachere E. Penaeidins, antimicrobial peptides with chitin-binding activity, are produced and stored in shrimp granulocytes and released after microbial challenge. J. Cell Sci, 2000;113: 461-469.
11. Bartlett TC., Cuthbertson BJ, Shepard EF, Chapman RW, Gross PS, Warr GW. Crustins, homologues of an 11.5 kDa antibacterial peptide from two species of penaeid shrimp, *Litopenaeus vannamei* and *Litopenaeus setiferus*. Mar. Biotechnol, 2002;4: 278-293.
12. Martin GC, Rubin N, Swanson E. *Vibrio parahaemolyticus* and *V. harveyi* cause detachment to the epithelium from the midgut trunk of the penaeid shrimp *Sicyonia ingentis*. Dis. Aquat. Org, 2004; 60: 21-29.
13. Lightner DV. Network of aquaculture centres in asia-pacific. Final Report, Asia Pacific Emergency Regional Consultation on the Emerging Shrimp Disease: Early Mortality Syndrome (EMS)/Acute Hepatopancreatic Necrosis Syndrome (AHPNS), Bangkok, Thailand, August, 2012.
14. Bhavan PS, Geraldine P. Histopathology of the hepatopancreas and gills of the prawn *Macrobrachium malcolmsonii* exposed to endsulfan. Aquat. Toxicol, 2000; 50: 331-339.
15. Verlhac V, Gabaudan J. Influence of vitamin C on the immune system of salmonids, *Aqua Fish Manage*, 1994; 25: 21.
16. Sakai M. Current research status of fish immunostimulants, *Aquaculture*, 1999; 172: 63.
17. Sahoo PK, Mukherjee SC. Immunomodulation by dietary vitamin C in healthy and aflatoxin B_1 induced immunocompromised rohu (*Labeo rohita*), *Comp Immunol Microbiol Infect Dis*, 2003; 26: 65.
18. Devasagayam TPA. Sainis KB. Immune system and antioxidants, especially those derived from Indian medicinal plants, *Indian J Exp Biol*, 2002; 40 639.
19. Prachumwat A, Thitamadee S, Sriurairatana S, Chuchird N, Limsuwan C *et al*., 2012. Shotgun sequencing of bacteria from AHPNS, a new

shrimp disease threat for Thailand. Poster, National Institute for Aquaculture Biotechnology, Mahidol University, Bangkok, Thailand.

20. Verri T, Mandal A, Zilli L, Bossa D, Mandal PK, Ingrosso L, Zonno V, Vilella S, Ahearn GA, Storelli C. D-Glucose transport in decapods crustacean hepatopancreas. Comp. Biochem. Physiol, 2001;130: 585-606.
21. Oanh, D.T.T., Hoa, T.T.T., Phuong, N.T., 2001. Characterization and Pathogenicity of Vibrio bacteria isolated from Freshwater Prawn (Macrobrachium rosenbergii) Hatcheries. Proceedings of the 2001 annual workshop of JIRCAS Mekong delta Project.
22. Oanh, D.T.T., Hoa, T.T.T., Phuong, N.T., 2001. Characterization and Pathogenicity of Vibrio bacteria isolated from Freshwater Prawn (Macrobrachium rosenbergii) Hatcheries. Proceedings of the 2001 annual workshop of JIRCAS Mekong delta Project.
23. Oanh DTT, Hoa TTT, Phuong NT. Characterization and Pathogenicity of Vibrio bacteria isolated from Freshwater Prawn (*Macrobrachium rosenbergii*) Hatcheries. Proceedings of the 2001 annual workshop of JIRCAS Mekong delta Project, 2001.