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Biochemical Changes and Growth Performance of Black Tigher Shrimp Larvae after using *Ricinus communis* extract as Feed additive

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Abstract: Shrimp seeds were fed with feed immersed in methanol extract of *Ricinus communis* in 20, 30, 40 and 50 % of the feed. The fifth group served as the control. Triplicate tanks were maintained for each group. This experiment was conducted for a period of 60 days the results were compared with control. At the end of culture the average growth of tank treated with group IV was 16.9 mm ± 0.31, but in control5.4 mm ± 0.5. At the end of experiment the Survival in group IV is 94.3% but in control 51.5%. The FCR of tank treated with group IV was very low (1.95 ± 0.05, control 3.66 ± 0.15). The total protein was found higher in animals fed with group IV (61.54± 7.44 %, control 45.01 ± 4.7 %). The total carbohydrate was higher in animals feed with group IV (33.54 ± 4.44 %, control group 26.1± 2.77 %). The protease activity was remarkably higher in group IV (123.54 ± 13.44Umg^{-1}, control 65.01 ± 8.77Umg^{-1} protein). The amylase activity was higher in group IV (113.54 ± 11.51Umg^{-1}protein, control66.12 ± 7.2Umg−1 protein). The lipase activity is higher in group IV (93.12 ± 8.4Umg^{-1}gut, control 59.34 ± 5.8Umg−1 gut). All these results were statistically found significant (P<0.05). It shows the 50 % methanol extract of *Ricinus communis* has better impact on shrimp Culture.
Key words: Aquaculture, Herbal extract, *Penaeus monodon, Ricinus communis,* Vibrio.

Introduction

The demand for animal protein for human consumption is increasing and it is met largely by terrestrial farmed animals. There has been a considerable increase in the culture of brackish water shrimp due to its high protein, less fat, taste, and market demand in both national and international markets. Thus, the research in the use of plant extracts for aquatic animals is increasing with the demand for eco-friendly and sustainable aquaculture, particularly for organic farming. Plants extracts decrease the selective pressure for developing antibiotics resistance[1].

The shrimp digestive system is activated particularly in the larval and early post-larval stages, where the plant extract would have the greatest effect[2]. The higher level of enzyme activity obtained with diets containing plant extracts improved the digestion of protein, carbohydrate, fat and cellulose, which might in turn explain the better growth observed with the shrimps fed with plant extract diets. Similar effects have been reported for fish and shrimp, in which digestion was shown to increase considerably in response to probiotics in the diet[3, 4,5].

This work aims to identify the growth rate and biochemical changes of shrimp tissue after using herbal extract as growth enhancer.

Materials and Methods

Collection and Preparation of Herbal extract

Live and healthy herbal plants *Ricinus communis* (Castor) were collected from different regions of Cuddalore district, India. It was washed twice using freshwater to remove epiphytes and other extraneous matter from the plants. Leaves of *Ricinus communis* (Castor), were shadow dried, well ground to make a fine powder. For solvent extraction, sieved powder was soaked with equal part of methanol (1:1), for 48 hrs[6].The slurry was then filtered and washed to remove non-soluble fractions. The filtered substance was then centrifuged (20,000gradient for 30 minutes).

The extracts were condensed at 35^0C, until the solvent residue has evaporated.

Experimental setup

The laboratory experiment was carried out in fiber tanks. Culture tanks were filled with sea water of 28 ppt. Twelve days old post larvae (PL 12) of *Penaeus monodon* (0.012 ± .001 mg), which were affected with Swollen Hind Gut obtained from commercial hatchery (Charoen Pokhapand aquaculture India Pvt. Ltd, Marakkanam). Shrimp *Penaeus monodon* were stocked in a cylindrical fiber tank (50 Liter capacity) in the laboratory. The post-larvae were acclimatized to laboratory conditions for 2 days prior to start of the experiment.

Experimental groups

After the acclimation period of 2 days, the shrimp were divided into five groups and stocked in the tanks at a stocking density of 10 numbers per tank. Four tanks were treated with feed immersed in methanol extract of *Ricinus communis* in 20, 30, 40 and 50 % of the feed. The fifth group served as the control. Triplicate tanks were maintained for each group. This experiment was conducted for a period of 60 days. The details are given below:

Group 1 = Basal diet + 20 % methanol extract of *Ricinus communis.*

Group 2 = Basal diet + 30 % methanol extract of *Ricinus communis.*

Group 3 = Basal diet + 40 % methanol extract of *Ricinus communis.*

Group 4 = Basal diet + 50 % methanol extract of *Ricinus communis.*

Control = Basal diet

Preparation of the feed

The commercial feed was mixed in the different concentration of the methanol extract of *Ricinus communis* and was allowed to dry. The feed ration was divided into 4 times a day as 25%, 20%, 30% and 25% in the morning (6.00AM), noon (12.00 PM) evening (6.00 PM) and night (10.00PM) respectively. Feeding rate was calculated from the average weight of shrimp for each period

Check trays were used to collect shrimps from each tank and wet weight was recorded on every 10 days. Survival was monitored at the end of the experiment. Shrimps were harvested after draining the tanks 60 days after stocking. Individual weight and survival of the tanks were also recorded.

Estimation of *Vibrio* load in the tanks and animal

Water and animal from experimental tanks were collected from each treatment tank. Animals were immersed in 50 mg l^{-1} formalin solution individually for 5 minutes (to remove the external bacteria present on the shrimps) and washed thoroughly using sterilized water for remove the remaining surface bacteria and disinfectant. The hepatopancreas and abdominal tissue samples were dissected aseptically and washed individually using sterilized water. The samples were then taken individually and homogenized with 5 ml of 85% saline and serially diluted the samples up to 10^{-5} dilution. This was done in the initial and final period of the experiment

Digestive enzyme production in the gut

The animal after the harvest were dissected and gut was separated. The separated gut samples were rinsed with cold distilled water. Total intestine content was then homogenized in phosphate buffer (pH 7.5; PBS) (1 g per 10 ml) using a hand held glass homogenizer at 4^o C. The homogenate was then centrifuged at 4^o C at 15000×g for 15 min. The supernatant was then stored at 4^o C prior to analysis. All enzymatic assays were conducted within 24 h after extraction.

Protease

To 3 ml of 0.6% casein solution (pH 9.0, prepared in 10Mm Tris-Hcl buffer) 0.5 ml of diluted enzyme solution was added and the reaction mixture was incubated at 45^o C for 10 min. The reaction was terminated by the addition of 3.2ml of TCA mixture (0.11M TCA, 0.22 M Sodium acetate, and 0.33 M acetic acid) and allowed to stand for 15 min before filtering through Whatman filter paper No.1. Aliquots of 0.5 ml were processed as per Lowrys *et al.,* (1951) methodology and the absorbance of 660nm. One unit of protease activity is expressed as the amount of enzyme which converts 1.0 mg of protein per 10 min at 45^o C. Protease enzyme activities were expressed as specific activity (U mg^{-1} protein)

Amylase

Amylase activity was measured according to Jiang(1982) and Worthington (1993) using iodine solution to reveal non-hydrolyzed starch. Amylase enzyme activities were expressed as specific activity (U mg^{-1} protein).

Lipase

Lipase activity was determined based on measurement of fatty acids release due to enzymatic hydrolysis of triglycerides in stabilized emulsion of olive oil[7,8] .Lipase activity was expressed as Ug−1 intestine content.

Estimation of protein

Protein was estimated by the method of Lowry *et al.,*

Reagents

Alkaline copper reagent:

Solution A: 2% sodium carbonate in 0.1N NaOH

Solution B: 0.5% copper sulfate in 0.1% sodium potassium tartarate

50 ml of the solution A was mixed with 1ml of solution B just before use.

Folin's Phenol reagent (1: 2 dilutions).

Bovine serum albumin (BSA).

Procedure

Pipette out 0.2 ml of standard Bovine serum albumin in a test tube and add 0.1 ml of extract, 0.7 ml of water and 4.5 ml of alkaline copper reagent were added and kept at room temperature for10 min. Then 0.5 ml of Folin's reagent was added and color developed was read after 20 min at 640 nm. The level of protein is expressed as mg g^{-1} tissue.

Estimation of Carbohydrate

Estimation of Carbohydrate was conducted by Phenol sulphuric acid

Materials

Phenol 5% : Redistilled (reagent grade) phenol (50g) dissolved in water and dilute to 1 liter .

Sulphuric acid: 96%reagent grade.

Standard glucose: stock – 100 mg in 100ml of water.

Working standard: 10 ml of stock diluted to 100 ml with distilled water.

Procedure

Weigh 100 mg of the sample in to a boiling tube**.** Hydrolyze by keeping it in a boiling water bath for three hours with 5ml of 2.5 N –HCL and cooled to room temperature. Neutralize it with solid sodium carbonate until the effervescence ceases. Make up the volume to 100 ml and centrifuge. Pipette out 0.2 ,0.4, 0.8 and 1ml of the working standard in to a series of test tubes .Pipette out 0.1 and 0.2 ml of the sample solution in to a separate test tubes. make up the volume in each tube to 1 ml with water . Set a blank with 1 ml of water. Add 1ml of phenol solution to each tube. Add 5 ml of 96 % Sulphuric acid to each tube and shake well. After 10 minutes shake the contents in the tubes and place in a water bath at 25- 30^{-0}c for 20 min. Read the colour at 490 nm. Calculate total amount total carbohydrate present in the sample solution using the standard graph .

Calculation

Absorbance cores tanks to 0.1ml of the test =x mg of glucose

100 ml of the sample solution contains

$$= \frac{X}{0.1} \text{ 100 mg of glucose}$$

= % of total carbohydrate present.

Statistical analysis

The results were analyzed using Student's *t*-test to determine the differences between tested groups. All statistics were performed with Duccan's Multiple Range Test (DMRT)by using the Statistical Package for Social Sciences (SPSS) for Windows, version 13.0 (SPSS Inc, Chicago, IL, USA).

Result

Survival

Survival rate in all the experimental tanks applied with herbal treatment were observed to be higher than the control tank. The results of't' test also showed significant survival ($P<0.05$) in all the treated tanks than the control tank. At the end of culture period the survival in tank treated with group IV was 94.3% followed by group II (89.3%). The survival rate of tanks applied with group III and group I was 84.3% and 73.5 % respectively. The survival was found to be very low (51.5%) in the tank kept as control .

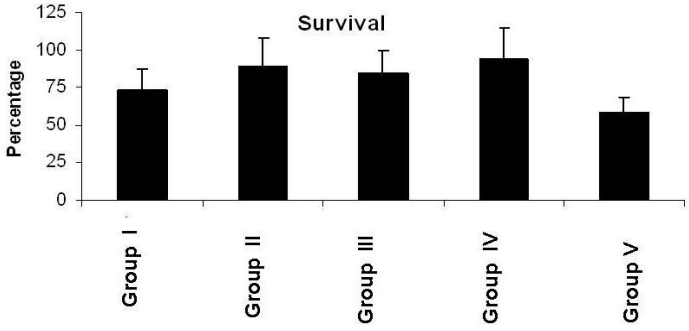

Fig.1 Survival of *P. monodon,* in the experimental and control tanks.

Growth

Maximum growth was observed in the tanks of group IV. Average weight gained by the shrimps in the group IV was around 20% greater than that of the control. The results of't' test also showed significant ($P<0.05$) of growth rate in all the treated tanks than that of the control tanks. At the end of culture the average growth of tank treated with group IV was 16.9 mm ± 0.31. This was followed by Group I (11.9mm ± 0.45), Group II (12.9mm ± 0.42) and Group III (13.5mm ± 0.37). The average growth was 5.4 mm ± 0.5 in the tanks kept as control without microbes.

Food Conversion Ratio (FCR)

The Food conversion ratio (FCR) was lower in the experimental tanks than the control tank and the difference was statistically found significant ($P<0.05$). After showing initial similarity, the FCR of the control tank was increased until the end of culture (fig.9). At the end of experiment the FCR of tank treated with group IV was 1.95 ± 0.05. This was followed by Group II (2.25 ± 0.06), Group 111(2.49 ± 0.08) and Group I (2.79±0.09). The FCR noted was 3.66 ± 0.15 in the tanks kept as control at the end of the culture.

Enzyme activities

At the end of the experiment, mean digestive enzyme activities (protease, amylase and lipase) in the gut of the experimental animal *P. monodon* collected from treatment tanks were significantly different ($P<0.05$) with that of the control. The protease activity was remarkably higher ($P<0.05$) in group IV (123.54 ± 13.44Umg^{-1}protein) and Group II (114.50 ± 13.90Umg^{-1} protein) compared with Group III (103.54 ± 10.44Umg^{-1}protein) and Group I (98.82 ± 10.38Umg^{-1} protein). Whereas the protease activity was noted as 65.01 ± 8.77Umg^{-1} protein in the gut of the animals collected from the tanks that was kept as control.

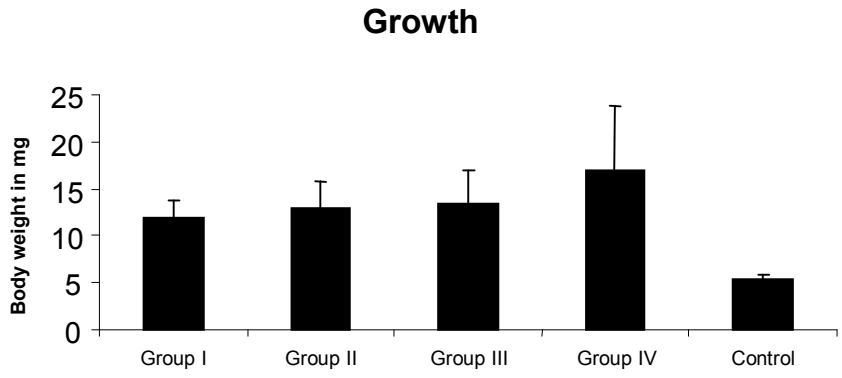

Fig.2 Average body weight of *P. monodon* in experimental and control tanks

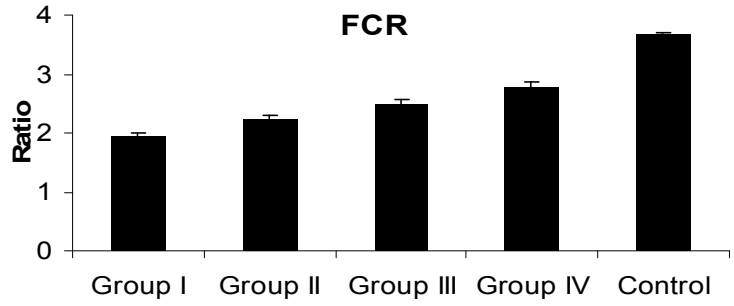

Fig.3 Food Conversion Ratio of *P. monodon* in control and experimental tanks.

A similar trend of activity was also found in the case amylase and lipase activity. The amylase activity was significantly higher (P<0.05) in group IV (113.54 ± 11.51Umg^{-1}protein), followed by Group II (109.10 ± 10.20Umg^{-1} protein) compared with Group III (99.14 ± 8.44Umg^{-1}protein) and Group I (96.12 ± 10.28Umg^{-1} protein). In control the amylase activity was estimated as 66.12 ± 7.2Umg−1 protein. The lipase activity was significantly higher (P<0.05) in group IV (93.12 ± 8.4Umg^{-1}gut), followed by Group II (89.10 ± 8.2Umg^{-1}gut) compared with Group 111(79.11 ± 6.2Umg^{-1}gut) and Group I (76.45 ± 6.1Umg−1 gut). In control tanks the lipase activity was estimated as 59.34 ± 5.8Umg−1 gut.

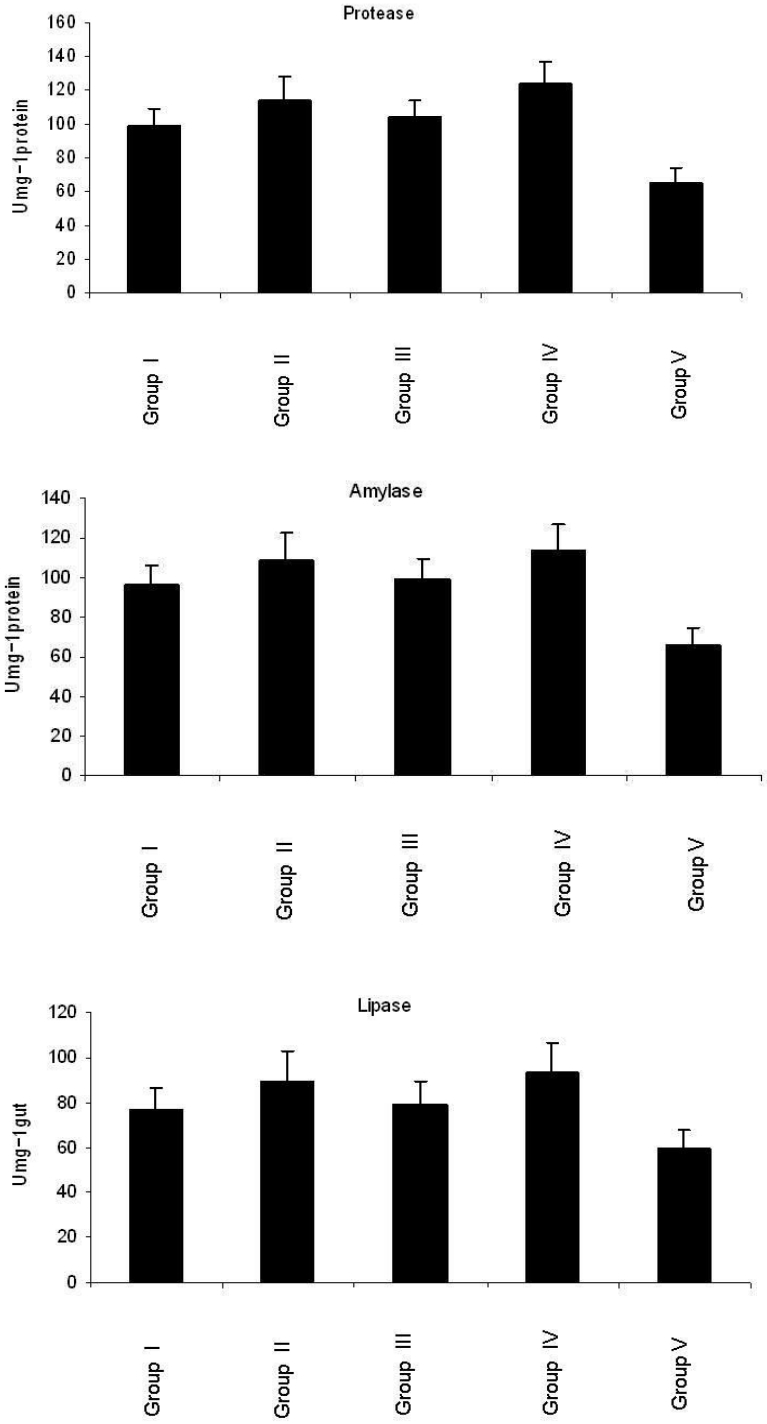

Fig.4 Changes in enzyme activities of *P. monodon* in experimental and control tanks.

Biochemical changes in the shrimp

Total protein

The total protein was found higher in animals fed with group IV (61.54± 7.44 %) followed by Group II and Group I (58.82 ± 6.38 % and 58.5 ± 6.38 %). In the animals collected from the Group III tank, the total protein was found to be (53.5 ± 6.44 %). The lowest quantity of protein was found in the animals from control tank (45.01 ± 4.7 %).

Carbohydrate

The total carbohydrate was higher in animals feed with group IV (33.54 ± 4.44 %) followed by Group II and Group III (29.4 ± 3.94 % and 29.1 ± 3.4 %). In the animals collected from the tanks treated with Group I, the total carbohydrate was estimated as (26.2± 3.38 %). This value was similar in case of the animals collected from tanks of control group 26.1± 2.77 %).

Vibrio load

The initial Vibrio load in the gut of the shrimp collected from all the tanks was 0.58× 10^4 CFU / ml In general, the Vibrio population in the gut of shrimp was higher than that of water in both control and treatment tanks. However, among the treated tanks, group IV was showing a reduced count of *Vibrio* spp. (0.14 × 10^4 CFU/ml). Among the herbal extract treated, Group I treated showed minimum (0.12 × 10^4 CFU/ml) followed by Group II (0.11× 10^4 CFU /ml) and Group III (0.09× 10^4 CFU/ml). In control it was found increased as 0.68× 10^4 CFU /ml

In water, the *Vibrio* spp. was found higher in the tanks kept as control (3.99 × 10^4 CFU /ml) at the end of the culture period. In general, the Vibrio population of sediment was higher than that of water in both control and treatment tanks. However, among the treated tanks, group IV was showing a reduced count of *Vibrio* spp. (0.64× 10^4 CFU /ml). Among the microbes Group II treated showed minimum (1.64 × 10^4 CFU /ml) followed by Group 111(2.54 × 10^4 CFU/ml) and Group I (2.51× 10^4 CFU /ml).

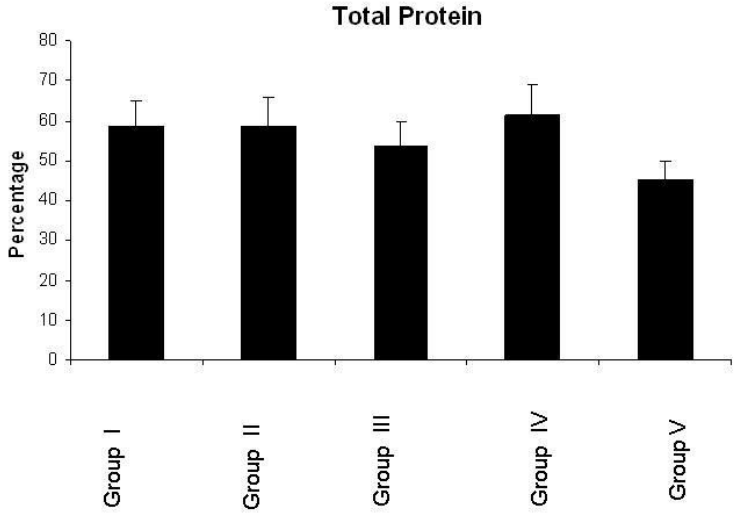

Fig.5 Changes in protein content of *P. monodon* in experimental and control tanks.

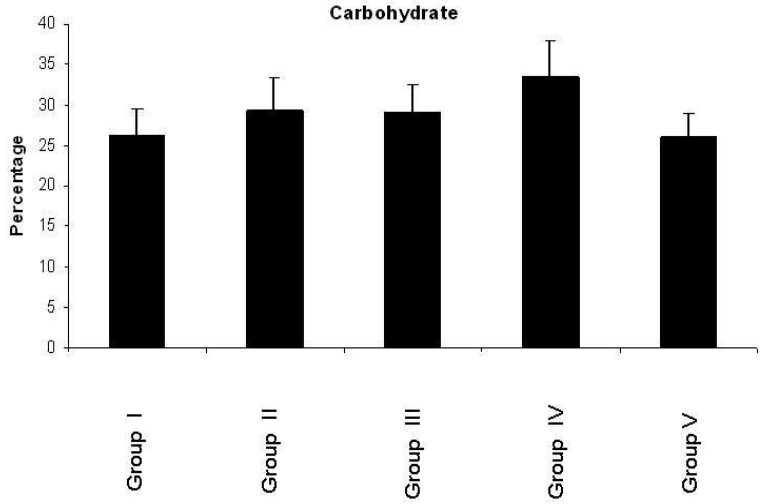

Fig.6 Changes in carbohydrate content of *P. monodon,* In experimental and control tanks.

Discussion

The abuse of antimicrobial drugs, pesticides, and disinfectants in aquaculture disease prevention and growth promotion has led to the evolution of resistant strains of bacteria and questions of safety[10,11]. Thus, the research in the use of plant extracts for aquatic animals is increasing with the demand for ecofriendly and sustainable aquaculture. Plants extracts decrease the selective pressure for developing antibiotics resistance[1] .

Ayurvedic herbal compounds are having potential effect on growth and survival as well as antimicrobial properties of aquatic organisms. In the present investigation five herbal plants such as *R. communis, P. niruri, A. lebbeck, C. asiatica* and *T. cordifolia* were tested.

The role of plant in growth rate was confirmed with production of the digestive enzymes like protease, amylase and lipase. The shrimps harvested at the end of the culture were then studied for biochemical changes. This revealed that shrimps that were treated with herbal extracts have more protein and carbohydrate.

To develop alternative practice for disease management in aquaculture, attention should be deviated to find novel drugs, especially from plant sources. Immanuel *et al*.[12], studied the shrimps fed with herbal and seaweed diets-enriched Artemia boosted the survival, specific growth rate and lowered *V. parahaemolyticus* load in muscle and hepatopancreas tissues in the culture system. In the present study the herbal plant, 50% *R. communis* mixed with basal diet showed better results than the others. Citarasu *et al*., (2002) describe the antimicrobial effects of herbal product that significantly increased the survival rate of tiger shrimp (*P. monodon*) larvae. Babu and Marian[13] demonstrated the effective spent- spanners utilization increasing fecundity and disease resistant larval production in *P. monodon* by using herbal supplemented diets.

The enhanced growth performance of shrimp might be due to increasing digestive enzyme activity induced by the plant extract. The shrimp digestive system is activated particularly in the larval and early post-larval stages, where the plant extract would have the greatest effect[2]. The higher level of enzyme activity obtained with diets containing plant extracts improved the digestion of protein, carbohydrate, fat and cellulose, which might in turn explain the better growth observed with the shrimps fed with plant extract diets. Similar effects have been reported for fish and shrimp, in which digestion was shown to increase considerably in response to probiotics in the diet[3,4,5] .In the present study, different concentrations of methanol extract of *R. communis* had different effects on enzyme activity, especially those of protease and amylase However, the activity of the digestive enzymes did not increase with increased concentration of plant extract. This suggests that the increased activity of the digestive enzymes in the shrimp intestine induced by extrinsic plant strains has an inherent limit. The general conclusion obtained from the present study is that the plant extract plays a vital role in growth, survival and disease resistance of the animal throughout the culture period. It is clear from the microbial load data that *Vibrio* spp is dominant in the control tanks.

Conclusion

The methanolic extract *R. communis* was applied in culture tanks of *P. monodon* with different dosages which is compared with control tank. Rate of survival and growth was found to be more in the experimental tank. Feed Conversion Ratio (FCR) and ammonia concentration were found lower in the plant extract applied tanks than the control tank. The bacterial population decreased at the end of culture in both experimental and control tanks. The load of *Vibrio spp* in the control tank was not showing any significant decrease. Hence it is clear that the methanolic extract of *R. communis* played a vital role in the growth, survival and disease resistance in the black tiger shrimp *P. monodon.*

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