



Effect of the Natural Herb Amla (*Phyllanthus emblica*) on Growth, Survival and Disease Resistance in *Macrobrachium rosenbergii* Juveniles

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Abstract

Phyllanthus emblica is a natural herb commonly known as Indian gooseberry or amla, belongs to the family Euphorbiaceae, found natively in India and all parts of this plant are used for several medicinal remedies in Ayurveda. Fruits are highly nutritious and rich source of vitamin C, amino acids and minerals and have tremendous pharmacological applications. In this study three experimental diets composed of ingredients such as fish meal, rice bran, Ground Nut Oil Cake (GNOC), wheat flour, vitamin and mineral mix and different quantities of amla (*P. emblica*) powder were fed to juveniles of *M. rosenbergii* (PL 16). Three experimental feed T1, T2 and T3 containing amla powder incorporated at the rate of 0.5 g, 1.0 g and 1.5 g was prepared and feed mixture without amla was used as control. General observation to be given here the juveniles fed with T3 showed maximum growth increment (1.92 ± 0.25 g) than T1 (1.15 ± 0.13 g) and T2 (1.72 ± 0.22 g). The mean growth increment with respect to length showed significant variations ($p < 0.005$) between treatments. The mean weight of individuals in T2 and T3 groups varied significantly compared to control. FCR values were found in T1 (1.19), comparatively better than T2 (1.59), T3 (1.66) and control (1.91). Whereas SGR values increased from 0.47 to 0.67 from control to T2. The highest percentage survival was recorded in T2 (100 ± 0) during study. In the bacterial challenge test against *Aeromonas hydrophila*, post larvae fed with T3 (1.5 g 100 g⁻¹) feed showed better survival percentage

after 72 h. All the results were statistically checked and significant ($p < 0.005$) between treatments.

Keywords: Amla, scampi, growth, feed

Introduction

Aquaculture expansion primarily depends on supplemental-fed systems. The high cost of nutrients (including manures and inorganic fertilizers), feed ingredients and formulated feeds are restricting supply of aquatic protein for aquaculture in many developing countries (Edwards & Allan, 2004). It is recognized that the lack of quality feeds is the major constraint to development of aquaculture production in cultured farming systems. The use of quality feeds has enabled farmers to culture valuable aquatic species for high profits. It is generally accepted that aquatic animals are more sensitive to feed quality when compared to terrestrial animals (Akiyama, 1992). A sound understanding of feed ingredient quality is the most critical factor in consistent production of high quality feeds. Variability in feed quality is always related to feed ingredient quality and its cost effectiveness (Glencross et al., 2007).

The giant freshwater prawn, *M. rosenbergii* has received considerable attention due to its nutritious delicacy to mankind (Poongodi et al., 2012). It is a prime aquaculture species, commonly called 'scampi' and has recently gained importance in south Asian countries after significant loss in penaeid shrimp culture after mid-1990s due to viral diseases (FAO, 2003). It has been reported that the world production of *M. rosenbergii* exceeded 4,00,000 tonnes in 2010, with a more than 20-fold increase compared to the early 90s (FAO, 2010). A comparison on global production of *M. rosenbergii* from 2010-16 reported that 6568 t in 2010 and reach up to 10152 t in 2016.

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Production declined in the year of 2011 (3721 MT), 2012 (4269 MT), 2014 (3545 MT) and value reached high in 2015 (7989 MT) was reported by MPEDA. In contrast to penaeid shrimps, *M. rosenbergii* is moderately disease-resistant (Farook et al., 2019). However, viruses such as *Macrobrachium* Hepatopancreatic Parvo-Like Virus (MHPV), *Macrobrachium* Muscle Virus (MMV), Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), White Spot Syndrome Virus (WSSV), *M. rosenbergii* Nodavirus (MrNV) and Extra Small Virus-Like Particle (XSV) have been reported and are responsible for economic losses to freshwater prawn culture (Hameed & Walker, 2009). The application of antibiotics and other chemicals to pond culture is also quite expensive and undesirable because of its environmental risks (Maqsood et al., 2011). In place of chemotherapeutic agents, focus now is on immunostimulants for disease control in aquaculture systems.

Medicinal plant extract are being widely used to minimize the use of antibiotics in fish health management (Chakrabarti & Vasudeva, 2006). Many studies have proved that herbal additives enhanced the growth of fishes besides protecting from them diseases (Francis et al., 2005; Ahilan, 2010; Direkbusarakom, 2004). Herbal additives in feed may enhance the digestibility and palatability of feed and improve the prawn health and maintain its product quality. Nowadays herbs are used for controlling shrimp and fish diseases through their immunostimulating functions and have growth promoting effects (Poongodi et al., 2012).

Indian herbs such as *Oscimum sanctum*, *Solanum trilobactum*, *Azadirachta indica*, *Phyllanthus emblica* are reported to have immunostimulating properties (Galina et al., 2009). *P. emblica* has antioxidant activity, anti-fungal activity, antimicrobial activity and anti-inflammatory activity. Amla fruit pulp contains large proportion of vitamin C, which has also been identified as an immunostimulant (Bairwa et al., 2012). Researchers studied the immunostimulatory potential of *P. emblica* in diet for promoting growth and hematology in *Tilapia mossambicus*, when challenged with *Pseudomonas aeruginosa* (Sivagurunathan et al., 2012). Using different concentrations of amla powder as feed additive on *Labeo rohita* fingerlings of Utharakhand, India and had promising effect on growth and survival (Tamta & Saxena, 2018). The present study was carried out to determine the growth perfor-

mance and disease resistance of *M. rosenbergii* by using *P. emblica* powder as a feed ingredient.

Materials and Methods

The post larvae of *M. rosenbergii* (PL 16 stage) were collected from a hatchery nearby the laboratory and were acclimatized for 7 days in two 100 L stocking tanks. The physico-chemical properties of water were maintained as normal (temperature 28°C, pH7 and dissolved oxygen 7 mg l⁻¹) during acclimatization. The post larvae were fed with crushed pellet feed at the rate of 15% of body weight. Four experimental diets were prepared ingredients procured from a local market. The details of composition of experimental diets are given in Table 1. Three experimental diets (T1, T2 and T3) were prepared with dried *P. emblica* powder at the rate of 0.5 g, 1.0 g, 1.5 g respectively. 100 L FRP round tanks were set for control and test experiments (T1, T2 and T3) each in triplicates and maintained in the hatchery premise of School of Industrial Fisheries, CUSAT. Ten post larvae, with average weight of 0.02 g were placed in each tank and were reared for 84 days. 25% of water was exchange twice in a week. Experimental and control feed were given in feeding trays provided at the centre of each tanks. Feeding was done two times daily. After 3 h, the uneaten feed and faecal matter were collected by siphoning which was later dried in oven at 40°C for 3 h, before recording weight. During experimental period, water quality parameters such as temperature, pH and dissolved oxygen were measured (APHA, 1995) daily.

Eighty four days of different experimental groups fed with *P. emblica* powder incorporated as 0.5 g 100 g⁻¹ (T1), 1.00 g 100 g⁻¹ (T2) and 1.5 g 100 g⁻¹ (T3). The ingredients were Fishmeal, Ground nut oil cake, Rice bran, Wheat flour @ ratio of 3:2:2:2 respectively.

At the end of growth studies, length and weight measurements, food conversion ratio (FCR = Feed consumed (g) X body weight gain (g)⁻¹) and specific growth rate (SGR = Log w_t - log w_o X 100 Days⁻¹) of experimental prawns were calculated.

Sample of cultured *Aeromonas hydrophila* obtained from National Centre for Animal Health, Lake Side Campus, CUSAT. Prawns from both treatment and control tanks were challenged with *A. hydrophila*. The bacterial stock was prepared from a single colony of *A. hydrophila* in nutrient agar plate by transferring to 2 ml nutrient broth a (NB) and incubating for

12 h which, then was diluted to 1 L NB and incubated overnight. After incubation, culture suspension was serially diluted to 10 fold with phosphate buffer saline (PBS). Bacterial concentration was counted as 9×10^6 CFU ml⁻¹. One litre of bacterial solution was transferred to tanks with 4 L water. After 3 h of bacterial treatment, the prawns were washed in a tank with dechlorinated water. Mortality in each group was checked for 7 days. 0.1 ml (one hundred microlitres) of haemolymph sample from one prawn in each experimental tank was taken before and after challenge test against *A. hydrophila*. Haemolymph samples were taken from behind the rostrum of prawn, using 1.0 ml syringe containing 0.9 ml anticoagulant sodium citrate and then transferred in to 1.5 ml micro tube. Haemocytes were counted using Neubauer haemocytometer. The total haemocyte count was recorded following the method of (Veile, 1990). One-Way ANOVA, Tukey and HSD analysis of the results were done using SPSS software. Significant treatment effect was separated by calculating the least significant difference at 5% level.

Result and Discussion

Control and the three experimental diets, T1 (0.5%), T2 (1%), T3 (1.5%) were prepared as small pellets having 5 mm size. The details of proximate composition of feeds are given in Table 2. The mean protein content of feed was ranged from 37.4% (T1) to 39% (C) while the same for total lipids ranged from 5% in C to 8.6% in T1. The average proximate value for moisture content were 4.3, 2.8, 4.8 and 5.5 g in Control, T1, T2 and T3 respectively. Similarly, the average ash contents were 5 g, 3 g, 2.55 g and 2.7 g in Control, T1, T2 and T3 respectively. However, the statistical analysis on proximate composition did not reveal any significant variation between experimental diets ($p > 0.005$).

The details of water quality parameters recorded in various tanks during the experiment are depicted in Table 3. Temperature during the culture period ranged from 30.0 to 30.8°C in all treatments. The mean values of temperature ranged from 30.2 $\pm$ 0.3 °C in T2 to 30.3 $\pm$ 0.3°C in T3. The pH ranged from 6.0 to 7.8 in all treatments while the lowest and

Table 1. Details of composition of different feeds prepared during the present study

Sl. No.	INGREDIENTS/100g OF FEED	CONTROL DIET (g)	EXP. DIETT 1 (g)	EXP. DIETT 2 (g)	EXP. DIETT 3 (g)
1	Fish meal	31.40	31.90	31.40	31.40
2	Ground nut oil cake	21.00	21.00	21.00	21.00
3	Rice bran	23.30	22.30	22.30	21.80
4	Wheat flour	23.80	23.80	23.80	23.80
5	Vitamin Mineral Mix	0.50	0.50	0.50	0.50
6	<i>Phyllanthus</i> powder	—	0.50	1.00	1.50
	TOTAL	100.00	100.00	100.00	100.00

Table 2. Proximate composition of various test feeds prepared (Mean $\pm$ SD)

	Control	Experimental feed (T1)	Experimental feed (T2)	Experimental feed (T3)
Protein	39.00 $\pm$ 0.00	37.40 $\pm$ 0.00	38.75 $\pm$ 1.25	38.51 $\pm$ 0.10
Lipids	5.00 $\pm$ 1.60	8.60 $\pm$ 3.60	10.47 $\pm$ 1.67	6.30 $\pm$ 1.58
Ash	5.00 $\pm$ 1.00	3.00 $\pm$ 0.00	2.55 $\pm$ 0.150	2.70 $\pm$ 0.00
Moisture	4.30 $\pm$ 0.30	2.80 $\pm$ 0.40	4.80 $\pm$ 0.20	5.50 $\pm$ 0.70

Note: The statistical analysis on proximate composition did not reveal any significant variation between experimental diets ($p > 0.005$).

Table 3. Mean $\pm$ SD of water quality parameters recorded in experimental tanks

	Control	T1	T2	T3
Temperature ($^{\circ}\text{C}$)	30.3 $\pm$ 0.3	30.3 $\pm$ 0.1	30.2 $\pm$ 0.3	30.3 $\pm$ 0.3
pH	7.0 $\pm$ 0.1	7.2 $\pm$ 0.3	7.2 $\pm$ 0.3	7.2 $\pm$ 0.3
DO (mg l ⁻¹)	8.59 $\pm$ 0.10	8.60 $\pm$ 0.04	8.50 $\pm$ 0.06	8.55 $\pm$ 0.10

Statistical analysis with ANOVA did not reveal any significant variations between treatments.

Table 4. Mean $\pm$ SD of final length, weight and survival recorded in experimental tanks

	Control	T1	T2	T3
Survival (%)	73.3 $\pm$ 5.8 ^a	86.7 $\pm$ 5.8 ^b	100.0 $\pm$ 0 ^c	90.0 $\pm$ 0 ^b
Final length (mm)	10.9 $\pm$ 6.9 ^a	12.4 $\pm$ 3.8 ^a	19.0 $\pm$ 5.8 ^b	19.6 $\pm$ 6.5 ^b
Final weight (g)	0.12 $\pm$ 0.09 ^a	0.13 $\pm$ 0.06 ^a	0.24 $\pm$ 0.06 ^b	0.20 $\pm$ 0.07 ^b

Note: Post hoc analysis by Duncan test revealed statistically significant difference of percentage survival in T2 with all other treatments. Further, the percentage survival recorded in T1 and T3 were also found to be different from control.

highest mean values recorded were 7.0 $\pm$ 0.1 and 7.2 $\pm$ 0.3 (T1 and T3 respectively). Dissolved oxygen ranged from 8.26 to 8.68 mg l⁻¹. The lowest and highest mean DO values were 8.50 $\pm$ 0.06 (T2) and 8.60 $\pm$ 0.04 (T1) respectively. Environmental fluctuation and feed wastage was the reason of varying water quality parameters during experimental period. However, statistical analysis did not reveal any significant variations between treatments. After 84 days growth experiment, length and weight of prawns were recorded and the details are given in Table 4. The mean final length showed statistically significant variation between treatments ($F = 5.31$ p d'' 0.005). The lowest mean length increment could be recorded in control (10.9 $\pm$ 6.9 mm) while the same in T1, T2 and T3 were 12.4 $\pm$ 3.8, 19.0 $\pm$ 5.8 and 19.6 $\pm$ 6.5 mm respectively. Similarly, the final weight recorded also varied significantly between treatments ($F = 5.18$, p d'' 0.005). The final weight ranged from 0.03 to 0.32 g and the lowest mean weight could be recorded in control (0.12 $\pm$ 0.09 g), whereas the highest could be recorded in T3 (0.24 $\pm$ 0.06 g). Post hoc analyses revealed that length and weight recorded in treatments T2 and T3 varied significantly with the same recorded in treatments C and T1. FCR values were computed at 1.91, 1.66, 1.59 and 1.19 in control, experimental diet T3, T2 and T1 respectively whereas SGR values computed were 0.47, 0.61, 0.67 and 0.63 respectively. The highest percentage survival was recorded in T2 (100.00 $\pm$ 0.0) (Table 4) while the same in T1 and

T3 were 86.67 $\pm$ 3.33 and 90.0 $\pm$ 0.0 respectively. The lowest survival could be encountered in control (73.33 $\pm$ 3.33%). Results of ANOVA revealed statistically significant variation between controls ($F = 21.83$). Post hoc analysis by Duncan test revealed statistically significant difference of percentage survival in T2 with all other treatments. Further, the percentage survival recorded in T1 and T3 were also found to be different from control.

After three months of growth study, challenge test was conducted for 7 days against *A. hydrophila* with *M. rosenbergii* post larvae fed with *P. emblica* powder incorporated feed. Post larvae in T3 only showed better percentage of survival rate after 72 h (Fig. 1). Complete mortality could be encountered in control, T1 and T2 with in 24 h, 48 h and 96 h respectively. Improved survival could be noticed in animals fed with feed containing *P. emblica* powder at the rate of 1 and 1.5%. The highest count could be recorded in T3 (3.19×10^6 cells/ml) while the same in control, T1 and T2 were 1.18×10^6 cells ml⁻¹, 0.70×10^6 cells ml⁻¹ and 0.49×10^6 cells ml⁻¹ respectively.

Note: Study of challenge test conducted in *M. rosenbergii* post larvae fed with *P. emblica* incorporated feed with different experimental feed. ANOVA revealed statistically significant variation between controls ($F = 21.83$). Post hoc analysis by Duncan test revealed statistically significant difference of percentage survival in T2 with all other treatments.

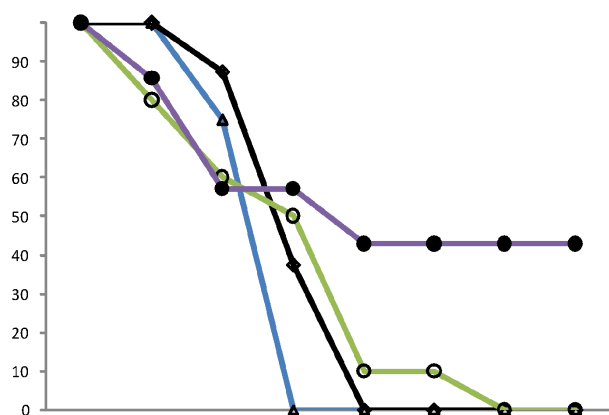


Fig. 1. Results of challenge test conducted in *M. rosenbergii* post larvae fed with *P. emblica* incorporated feed

Herbs are recently important and increased interest in aquaculture because of its immunostimulating function (Govind et al., 2012), immunologically active compounds such as polysaccharides, organic acids, alkaloids, glycosides and volatile oils, which can enhance immune functions. Plant extracts have been reported to favor growth promotion and immunostimulation properties in fish and shrimp aquaculture (Reverter, 2014).

The results of this experiment showed that incorporation of a suitable immunostimulant in supplementary feed is necessary to enhance growth of larvae at early stage. In the present study mortality was significantly reduced during experimental period. Further, lowest mean growth increment could be recorded in Control (0.97g) without using *P. emblica* (0.100 g^{-1}), and highest in T3 (1.92 g) was the incorporation of *P. emblica* ($1.5 \text{ g } 100 \text{ g}^{-1}$) also enhanced better growth compared to control and less mortality in experimental feeds [T1 ($0.5 \text{ g } 100 \text{ g}^{-1}$), T2 ($1.00 \text{ g } 100 \text{ g}^{-1}$) and T3 ($1.5 \text{ g } 100 \text{ g}^{-1}$)]. Use of *P. niruri* at 1.5 % concentration in supplementary feeds of gold fish yielded a highest mean weight gain (1.769 g) while the use of aloe vera at a concentration of 1% recorded a mean weight gain of 1.389 g (Ahilan et al., 2010).

The use of probiotics (Biogen®) in *M. rosenbergii* post larvae has also been reported to have significantly enhanced growth performance (Saad et al., 2009). A comparative growth study conducted on seaweed *Kappaphycus alvarezii* incorporated feed and a commercial feed in *P. monodon* grow out ponds at Diu region of Saurashtra coast reported to have 89.70% survival rate and average daily growth

(0.277 g) (Anil et al., 2011). Wild satavari (*Asparagus racemosus*) was used growth promoting agent in *Labeo rohita* fry (Direkbusarakom, 2004). Further, use of bitter kola *Garcinia kola* dry seed powder growth was found to promote growth in African sharp tooth catfish *Clarias gariepinus* fingerlings (Dada & Oviawe, 2010).

In the present study, FCR values were computed at 1.91, 1.66, 1.59 and 1.19 in control, experimental diet T1, T2 and T3 respectively. (Anil et al., 2011) reported better FCR values from seaweed *K. alvarezii* incorporated feed for penaeid shrimp in Pond 1 (1.52 : 1 and Pond 2 (1.26 : 1). Comparable FCR values have also been reported in Pacific White Shrimp (*Litopenaeus vannamei*) with yeast cells (Chotikachinda et al., 2008) where FCR ranged from 2.29 to 1.75. However, experiments with turmeric (*Curcuma longa* Linn.) extract incorporated feeds registered no significant variations in FCR values in *L. vannamei* (Lawhavinit et al., 2011). Deng et al., (2014) reported effect of polysaccharides from mycelia of *Cordyceps sinensis* (PMCS) as a feed additive for pacific white shrimp (*Litopenaeus vannamei*) the FCR value of PMCS group was 2.60, higher than that of control (2.37). Recent studies on medicinal herbs *Cissus quadrangularis* incorporated feed (5 % > 3 % > 1 %) on *M. rosenbergii* post larvae yielded maximum growth and nutritional indices, followed by *Eclipta alba* (5 % > 3 % > 1 %) and *Andrographis paniculata* (3 % > 5 % > 1 %). Among three herbs used *C. quadrangularis* incorporated feed fed PL showed the best performance followed by *E. alba* and *A. paniculata* in the above mentioned trend when compared with control ($p > 0.05$) (Radhakrishnan et al., 2014).

In the present study, high SGR values could be recorded with *P. emblica* incorporated feeds (T1, T2 and T3) compared to control group. Chotikachinda et al. (2008) reported similar results on yeast cells showing better SGR on experimental feeds compared to control feed in Pacific White Shrimp (*Litopenaeus vannamei*). Another Experiment conducted on herbs such as *Cissus quadrangularis*, *Eclipta alba*, *Alternanthera sessilis* incorporated feed in *M. rosenbergii* PL for 90 days show the SGR values were significantly higher in experimental diet compared to control (Radhakrishnan et al., 2014).

It could be stated that highest percentage survival was recorded in all test trials (T1 86.7%, T2 100% and T3 90%) after eighty four days of *P. emblica*

incorporated feed on growth study in contrast to the lowest survival was observed in control (73.33%). After eighty four days of growth study and 7 days of challenge test with *A. hydrophila* on *M. rosenbergii* juveniles showed better survival rate with *P. emblica* incorporated feed in T3 (1.5 g 100 g⁻¹) after 72 h. Animal feed with control diet has recorded complete mortality after 24 h of challenge study, T1 group fed with *P. emblica* (0.5 g 100 g⁻¹) showed maximum mortality after 48 h, whereas T2 group fed with *P. emblica* (1.00 g 100 g⁻¹) has recorded complete mortality after 96 h. Another study on different frequencies of marine *Vibrio alginolyticus* on juvenile *Penaeus monodon* against WSSV had similar results (Rohini et al., 2012). On the contrary, effects of ethanol turmeric (*Curcuma longa* Linn.) extract against shrimp pathogenic *Vibrio* spp. in *L. vannamei* revealed no significant difference ($P > 0.05$) in survival rate (Lawhavinit et al., 2011). After 60 days growth studies and 10 days of challenge with *A. hydrophila*, *Carassius auratus* fingerlings registered highest survival rates when fed on *P. niruri* and *Aloe vera* incorporated feeds (70 and 80 % respectively) (Ahilan et al., 2010).

P. emblica fruits have been shown to stimulate immune system of human beings and animals through enhanced phagocytosis, production of reactive oxygen and antigen species. It is rich in vitamin C, a potential immunostimulant and the antioxidant properties of vitamin C is well documented in shrimp (Darvishpour, 2012; Khopde, 2001). Charoenteeraboon, 2010). In the present study, improved survival could be noticed in animals fed with feed containing *P. emblica* powder @ 1 and 1.5 g 100 g⁻¹, after challenging with *A. hydrophila*.

In the present study, THC was found to vary from 0.49×10^6 cells ml⁻¹ to 3.19×10^6 cells ml⁻¹. Highest values of THC could be recorded in juveniles collected from treatments T2 to T3 which proved increased availability of defence cells in them. These findings may be corroborated with the high survival and increased disease resistance recorded in *M. rosenbergii* fed with *P. emblica* extract incorporated feeds. Therefore, the present study concludes that in addition to the benefit of enhanced growth rate and improved survival, incorporation of *P. emblica* in supplementary feeds at levels above 1 g 100 g⁻¹ of body weights can effectively increase disease resistance and immunity.

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