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# Influence of Ethanolic Extract of *Phyllanthus amarus* on the Growth of the Prawn *Macrobrachium rosenbergii* Post-Larvae

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## Abstract

The ethanolic extract of *Phyllanthus amarus* incorporated with artificial feed was found to produce better survival, growth, nutritional indices (weight gain and specific growth rate), activities of digestive enzymes (protease, amylase and lipase), and concentrations of basic biochemical constituents (total protein, amino acid, carbohydrate, and lipid) in *M. rosenbergii* post larvae when compared with control. The FCR was found to decrease in test prawns, which reflects the better quality of the feed offered. This study suggests that *P. amurus* have the potency to enhance the general health of test prawn. Thus, this herb is recommended as a feed additive for sustainable culture of *Macrobrachium*.

**Keywords:** Prawn; *P. amurus*; Survival; Growth; Health; Nutrition.

## 1. Introduction

The increasing demand and rising prices of seafood are raising the profile of freshwater prawn as an important aquaculture commodity. The genus *Macrobrachium* is commonly grown in almost every continent. The farming of the giant river prawn *Macrobrachium rosenbergii* popularly known as 'scampi' is a profitable industry in the world aquaculture sector (FAO, 2013). The existing culture system includes both monoculture and polyculture with Indian major carps in ponds. The recommended grow out stocking densities are range from 0.5-2.5 scampi per $m^2$ in polyculture and 1-5 per $m^2$ in monoculture. The newly-hatched *M. rosenbergii* larvae are normally start feeding about 1 day after hatching and initially require live feed. After a week they usually feed on combination with prepared diets, since this is an omnivorous (Dhont *et al.*, 2010). In grow-out stage, they are usually feed on farm-made or commercial feeds (Mitra *et al.*, 2005; Soundarapandian and Ananthan, 2008; Valenti, 1990). The use of good quality artificial feed, however, is important when biomass in the ponds increase as the animals grow. This is to achieve more uniform production of large prawns (Tidwell *et al.*, 2004). The artificial feeds are major concerns for farmers, representing up to 60% of the total variable production costs (Akiyama *et al.*, 1992; Sarac *et al.*, 1993). It must full-fill several characteristics, such as odor, texture, and flavor. In addition, it should be readily available at low cost, highly digestible with all the essential nutrients, such as protein, amino acids, carbohydrate, lipid, fatty acids, vitamins and minerals at desirable level for assimilation, and devoid of anti-nutritional factors (Smith *et al.*, 1992; Sudaryono *et al.*, 1995).

In aquaculture, hormones, antibiotics, vitamins and several other growth promoting chemicals have been used, but they have residual effects (Jayaprakas and Sambhu, 1996; Sambhu, 1996). The consumer awareness and concern over food safety have led to the search for alternative growth promoters of natural origin. It is very obvious that herbal preparations are of great medicinal interest due to the presence of phytochemical compounds, which are responsible for the biological activities they exhibit. The herbal products used in the aquaculture operations have the characteristics of growth promoting ability, tonic to improve the immune system, anti-microbial capability, anti-stress characteristics and stimulating appetite due to presence of alkaloids, flavonoids, pigments, phenolics, terpenoids, starch, steroids and essential oils (Citarasu *et al.*, 2002; Citarasu, 2010; Fukumoto and Mazza, 2000; Kamboj, 2010; Sivaram *et al.*, 2004). Fishes and prawns when fed with herbal supplemented feeds have resulted in increased weight gain, feed conversion efficiency and specific growth rate (Bhavan *et al.*, 2011; Bhavan *et al.*, 2012; Bhavan *et al.*, 2013a; Bhavan *et al.*, 2013b; Bhavan *et al.*, 2014a; Bhavan *et al.*, 2014b; Dhanalakshmi *et al.*, 2016;

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Kanagarasu *et al.*, 2017; Manjula *et al.*, 2018; Poongodi *et al.*, 2012; Radhakrishnan *et al.*, 2013; Shanthi *et al.*, 2012; Wu *et al.*, 2000).

Therefore, in the present study, ethanolic extracts of a medicinal plant, *Phyllanthus amarus* was incorporated with basal diet at 0.5% and 1.0% levels and fed to *M. rosenbergii* PL for 60 days for assessing its growth promoting ability. Under this background, the nutritional indices [survival rate (SR), length, weight, weight gain (WG), specific growth rate (SGR), food conversion ratio (FCR) and protein efficiency ratio (PER)], activities of digestive enzymes (protease, amylase and lipase), and contents of basic biochemical constituents (total protein, amino acid, carbohydrate and lipid) were studied.

# 2. Materials and Methods

## 2.1. Phyllanthus amarus

*Phyllanthus amarus* is a small erect annual herb (10-60 cm tall) with numerous small oblong-elliptic leaves and glabrous (6-12 mm long). The main stem is simple or branched, terrete smooth or scabridulous in younger parts**.** Flowers are very small, yellow in colour and hang down in beautiful array hidden below the leaves. The flowers produce very small (2 mm) fruits that burst open and the seeds are hurled away. When the plants are picked, the feathery leaves fold in, completely closing themselves. *P. amarus* is a common pantropical weed that grows well in moist, shady and sunny places. It is widely spread throughout tropics and subtropics (**Figure 1**)**.**

**Fig-1.** The medicinal herb, *Phyllanthus amarus* Linn

## 2.2. Preparation of Ethanolic Extract of P. amarus Whole Plant

*P. amarus* was collected from Bharathiar University campus, Coimbatore, Tamil Nadu, India (11° 2' 20.4792" N and 76° 52' 35.1084" E) and authenticated with Botanical Survey of India, Coimbatore, Tamil Nadu, India. The herb was thoroughly washed with freshwater, blotted and spread out and dried for two weeks at room temperature. Shade dried herb was ground to fine powder. *P. amarus* powder (50 g) was taken and packed in Whatmann No. 1 filter paper, and placed into soxhlet apparatus and soaked with 300 ml (1:6 w/v) of ethanol (99.9% purity, ChangshuYangyuan Chemicals, China, and sequentially extracted for 9 h (36 cycle). Repeated extraction was done until a clear colourless solution was obtained. The extracts were filtered by using double layer muslin cloth, and concentrated at 40-50 °C using rotary vacuum evaporator (ROTAVAP). The extract obtained was vacuum-dried under 40 °C and appeared as dark green, gummy solid**.** Its primary and secondary phytochemicals was characterized by us previously (Kalaiselvi *et al.*, 2018).

## 2.3. Preparation of Artificial Diet

The basal ingredients, such as fish meal, groundnut oilcake, soybean meal, wheat bran, tapioca flour, sunflower oil and hen egg were purchased from local merchants at Coimbatore. Vitamin B-complex with vitamin-C (Pfizer Ltd., Mumbai, India) was purchased from local medical shop. Each vitamin capsule contains, Thiamine Mononitrate IP, 10 mg; Riboflavin IP, 10 mg; Pyridoxine Hydrochloride IP, 3 mg; Vitamin B12 (as tablets 1:100) IP, 15 mcg; Niacinamide IP, 100 mg; Calcium pantothenate IP, 50 mg; Folic acid IP, 1.5 mg; Biotin USP, 100 mcg; Ascorbic acid IP, 150 mg.

The basal ingredients were pulverised separately using a micro pulverizer and hand sieved through ingredient siever. The sieved ingredients were weighed (fishmeal, 25%; groundnut oil cake, 25%, soybean meal, 25%, wheat bran, 10%; tapioca flour, 5%), mixed well and stream cooked for 15 min at 95-100 °C and allowed to cool at room temperature.With this, egg albumin (7%), vitamin B-complex with vitamin C (1%) and sunflower oil (2%) were added. The ethanolic extract of *P. amarus* was separately incorporated at 0.5% and 1% levels. The dough was prepared with adequate quantity of boiled water, pelletized in a manual pelletizer fixed with 3 mm diameter die, collected in stainless steel tray, and air dried until the moisture content reached less than 10%. The pellet was physically examined for visual appearance, such as uniformity, color and fragrant smell. The smooth surfaced pellet prepared with basal diet without incorporation of the ethanolic extract of *P. amarus* was served as control feed, which was subjected to proximate composition analyses (**Figure 2**).

**Fig-2.** Texture of control feed formulated using basal ingredients

## 2.4. Proximate Composition of Basal Diet Formulated

### 2.4.1. Determination of Moisture Content

The moisture content was determined by drying the sample in hot air oven as described in AOAC (1995). Feed sample (10 g) was taken on pre weighed concave glass and they were kept in desiccators, maintaining 0.5% relative humidity, dried at 105 °C for 24 h in the desiccators until they reached a constant weight.

$$\text{Moisture (\%)} = \frac{\text{Fresh sample weight (g)} - \text{Dry sample weight (g)}}{\text{Fresh sample weight (g)}} \times 100$$

### 2.4.2. Crude Protein Analysis

The protein estimation was done based on the conversion of organic nitrogen to inorganic nitrogen followed by Micro Kjeldahl's method (AOAC, 1995). Feed sample (10 g) was transferred into 250 ml of digestion tube, then 10 to 12 ml sulphuric acid with 0.2 g of digestion mixture (Potassium sulfate, anhydrous sodium sulfate and copper sulfate in the ratio of 9:1:1) were added and digested in a digestion chamber until a clear digest or colourless or slight green colour was obtained. After cooling, the volume was made up to 100 ml with distilled water. Then, 5 ml of digested solution was taken for distillation along with 10 ml of 40% sodium hydroxide solution. The liberated ammonia was absorbed in 2% boric acid solution containing mixed indicator (ethyl red and ethylene blue are dissolved in ethyl alcohol). Then, the boric acid solution was titrated against N/70 standard hydrochloric acid solution until the boric acid solution turned pink. Total nitrogen was calculated and expressed as g/100 g of sample. Protein content was obtained by multiplying nitrogen content with a factor 6.25.

$$\text{Total nitrogen (\%)} = \frac{\text{Nitrogen factor (14)} \times 0.1\ (0.1\text{N HCl}) \times \text{Burette reading}}{\text{Sample weight (g)} \times 1000} \times 100$$

Crude protein (%) = Total nitrogen × 6.25 (Protein conversion factor)

### 2.4.3. Crude Fibre Analysis

The crude fibre of feed sample was estimated as described by AOAC (1995). Feed sample (10 g) was digested with 0.128 M $H_2SO_4$ with a 2 or 3 drops of octanol in digestion unit (Hot extractor, Model-1017) for 30 minutes. Filtered and washed with hot water to remove acid, further residue was boiled with 0.223 M KOH for 30 minutes, then rinsed with boiling water and acetone. The residue was dried in an oven at 130 °C for 2 h and ignited in muffle furnace at 500 °C for 3 hours. The loss of weight represented the crude fiber.

$$\text{Crude fibre (\%)} = \frac{(A\text{-}B) \times 100}{C}$$

Where,

A = weight of crucible with dry residue (g).
B = weight of crucible with ash (g).
C = weight of sample (g).

### 2.4.4. Etheric Extract or Crude Fat Analysis

The crude fat content of moisture free feed sample was determined by extracting the fat with petroleum ether by using Soxhlet apparatus as described in AOAC (1995). Briefly, 10 g of moisture free feed sample was taken in an extraction thimble and it was placed in the extractor with an attached receiving flask. The solvent was poured into the thimble through a glass funnel. The receiver containing petroleum ether was heated at 40 to 60 °C, and the ether drops from the condenser to the thimble at the rate of 5 to 6 drops per second. When sufficient solvent was transferred to the extracting tubes to fill the siphon arm, it siphoned back into the receiver. This process was continued until the extraction was completed (around 16-18 hrs). After that, the flask was removed and the volatile solvent was evaporated at 60 to 80 °C on a rotary flash evaporator. The residue was dried in an oven and cooled in a

desiccator and weighed. The least weight of residue gives the weight of fat in the sample. The fat content of the sample was expressed on wet weight basis as percentage.

The crude fat content of the samples was calculated as:

Crude Fat (%) = (W3 - W2) × 100 / W1 × Lab DM / 100

Where,

W1 = initial sample weight in grams

W2 = tare weight of beaker in grams

W3 = weight of beaker and fat residue in grams

### 2.4.5. Determination of Ash Content

The ash content of feed sample was estimated as per AOAC (AOAC, 1995). Briefly, moisture free feed sample (10 g) was taken in pre-weighed crucible and incinerated in a muffle furnace at a temperature of 600 °C for 4 to 5 hours. Then the crucible was removed from the muffle furnace, allowed to cool in a desiccator. The weight of the crucible was taken and the value was expressed on wet basis as percentage.

$$\text{Ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Sample weight (g)}} \times 100$$

### 2.4.6. Determination of Total Nitrogen Free Extract

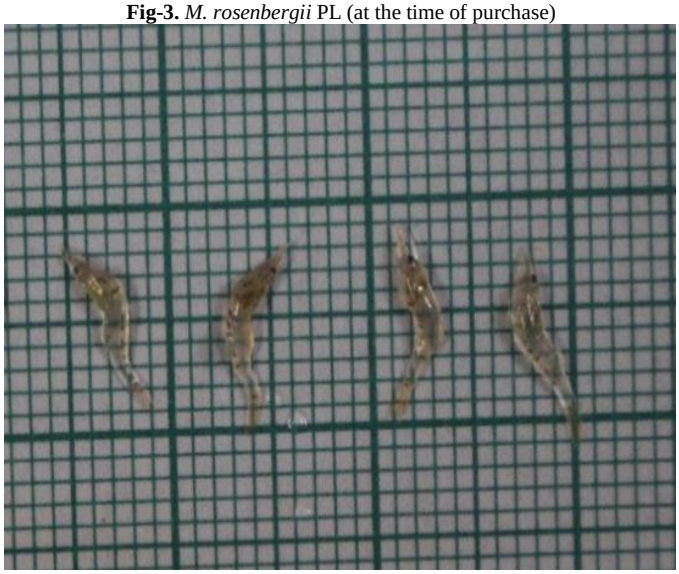
The proximate composition of total nitrogen free extract (TNFE) was calculated as described by Castell and Tiews (1980), by subtract the contents (%) of the following five parameters, moisture, crude protein, ether extract, crude fiber and ash.

NFE (%) = 100 - (% crude protein+ % crude fibre + % crude fat + % ash + % moisture)

### 2.5. Procurement and Acclimatization of Experimental Animal

The early post larvae (PL-20) of the freshwater prawn, *M. rosenbergii* (0.8±0.06 cm in length and 0.06±0.02 g of weight) were procured from Sri Durgai Hatcheries, Chengalpattu, Tamil Nadu, India (12°15' and 13°15' N and 79°15' and 80°20' E) (**Figure 3**). They were transported to the laboratory in polythene bags filled with oxygenated water. The prawns were acclimatized to the ambient laboratory condition with ground water in cement tanks (6 × 3 × 3 feet) for two weeks (temperature, 26.5±1.3 °C; pH, 7.01±0.12; TDS, 0.80±0.05g/l; DO, 7.30±0.49mg/l; BOD, 29.0±1.24mg/l; COD, 124.0±3.2mg/l; ammonia, 0.026±0.007 mg/l). During acclimatization the PL(s) were fed with *Artemia* nauplii, egg albumin and Scampi crumble feed alternatively. At that time it reached 1.6±0.2 cm in length and 0.6±0.1g of weight. About half of the tank water was renewed each day and adequately aerated to maintain a healthy environment. This ensures an environment devoid of accumulated metabolic wastes and sufficient supply of oxygen. The unfed feeds, faeces, moult and dead prawns were siphoning-out daily.

**Fig-3.** *M. rosenbergii* PL (at the time of purchase)



### 2.6. Feeding Trails with Ethanolic Extract of *P. amarus* Incorporated Diet

*M. rosenbergii* PL ranging from 1.6 ± 0.2 cm in length and 0.6 ± 0.1g in weight were used in this experiment**.** The prawns were divided into three groups. One group served as control and fed with basal diet formulated, and the other two groups were fed with ethanolic extract of *P. amarus* (0.5% and 1.0%) incorporated diets for 60 days in a triplicate experimental set up. The feeding was allowed two times per day (8.00 and 20.00 hrs) at 10% of body weight. The water medium was renewed daily without severe disturbance to the prawn and aerated adequately. At the end of the experiment, the final length and weight were measured for calculating the nutritional indices (**Figures 4-6**). The activities of digestive enzymes were assayed, and the contents of basic biochemical constituents were estimated.

**Fig-4.** *M. rosenbergii* PL fed with control diet at the end of the feeding trial

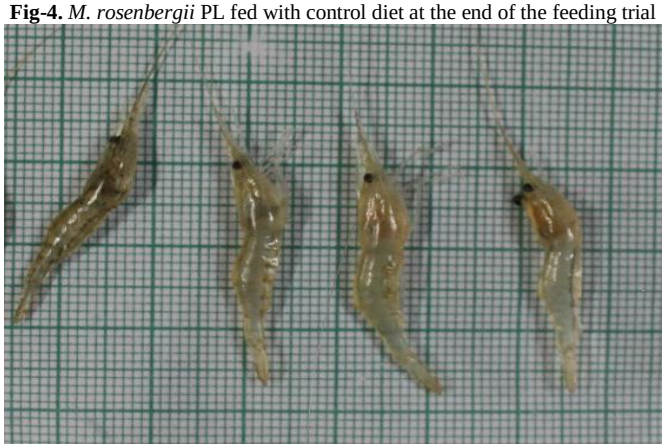


**Fig-5.** *M. rosenbergii* PL fed with 0.5% of ethanolic extract of *P. amarus* incorporated diet

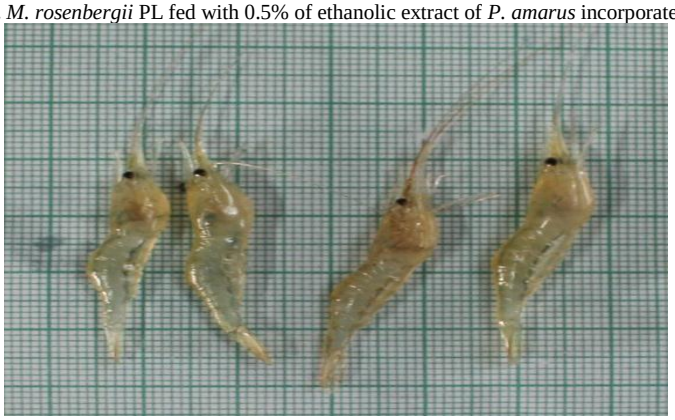
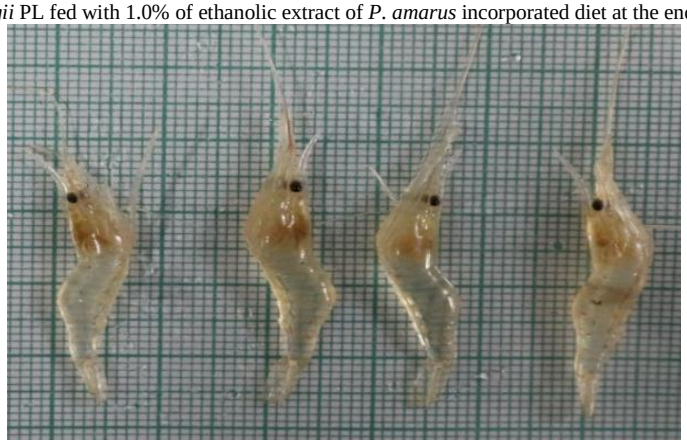


**Fig-6.** *M. rosenbergii* PL fed with 1.0% of ethanolic extract of *P. amarus* incorporated diet at the end of the feeding trial



### *2.6.1. Determination of Nutritional Indices*

Nutritional indices, such as survival rate (SR), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency rate (PER) were determined by following equations (Tekinay and Davies, 2001).

i. Survival (%) = Total No. of live animals/Total No. of initial animals × 100
ii. Weight gain (g) = Final weight (g) – Initial weight (g)
iii. Specific growth rate, (%) = $log\ W_2 - log\ W_1/t \times 100$

Where, $W_1$ &$W_2$ = Initial and Final weight respectively (g)**,** and t= Total number of experimental days

iv. Food conversion ratio (g) = Total Feed intake (g)/ Total weight gain of the prawn (g)
v. Protein efficiency ratio (g) = Total Weight gain of PL (g)/ Total Protein consumed (g)

### *2.7. Assays of Digestive Enzymes*

Activities of digestive enzymes were assayed at '0'day and at 60$^{th}$ day of feeding trial. The digestive tract of three prawns from each replicate were carefully dissected and homogenized in ice-cold distilled water and centrifuged at 9000 g under 4 ºC for 20 min. The supernatant was used as a source of crude enzyme. Total protease activity was determined by casein-hydrolysis method of Furne *et al.* (2005), where one unit of enzyme activity represented the amount of enzyme required to liberate 1 µg of tyrosine per minute. Amylase activity was determined according to Bernfeld (1955), the specific activity of amylase was calculated as milligrams of maltose liberated per gram of protein per hour (mg/g/h). Lipase activity was assayed by the method of Furne *et al.* (2005), one unit of lipase activity was defined as the amount of free fatty acid released from triacylglycerol per unit time.

### 2.8. Estimations of Basic Biochemical Constituents

The initial and final concentrations of total protein, amino acid and carbohydrate in experimental PL were estimated by adopting the methodology of Lowry *et al.* (1951), Moore and Stein (1948) and Rao and Gurfinkel (2000), respectively. The total lipid was extracted by Folch *et al.* (1957) method, and estimated gravimetrically by Barnes and Blackstock (1973) method. The contents of ash and moisture were analysed by following AOAC (1995) methodology were estimated.

### 2.9. Statistical Analysis

Data between control versus experiments and between experiments were subjected to statistical analysis through one-way ANOVA and subsequent post hoc multiple comparison with DMRT by adopting SPSS (v20). All the details of statistical analyses were given in respective tables. The P values less than 0.05 were considered statistically (95%) significant.

## 3. Results and Discussion

### 3.1. Proximate Composition of Basal Diet Formulated and Feed Quality

The proximate composition of basal (control) diet formulated contains 11.43% moisture, 43.77% crude protein, 1.43% crude fibre, 7.19% crude fat (etheric extract), 6.91% ash, 34.85% total carbohydrate (total nitrogen free extract), and 4163 kcal/kg gross energy (**Table 1**). It satisfied the prescribed quantum of protein (35-40%), carbohydrate (25-35%) and fat (6-8%) for 'Scampi'culture (Mitra *et al.*, 2005; Rangacharyulu, 1999; Swamy, 1995). It also satisfied the required energy value (3400-4000 kcal/ kg). In this study, the fish meal, groundnut oilcake, and soybean meal were taken as protein sources. The wheat bran was used as carbohydrate source. The sunflower oil was taken as lipid source. The egg albumin and tapioca flour were used as binding agents. The micro nutrients like vitamin B-complex were used as they are essential for animal growth. This type of simple feed formula is suitable for on farm feed management.

**Table-1.** Proximate composition of basal diet formulated

| Composition | Quantity (%) |
|---|---|
| Moisture | 11.43 |
| Crude Protein | 43.77 |
| Crude Fibre | 1.43 |
| Crude fat | 7.19 |
| Total Ash | 6.91 |
| Total carbohydrate | 34.85 |
| Gross Energy | 4163 kcal/kg |

Protein is essential for growth and development as it provides the body with energy and is needed for the production of hormones, antibodies, enzymes and tissues. The gross dietary protein requirement is influenced directly by the amino acid composition of the diet (Wilson, 2002). In aqua feeds, protein is the most expensive energy component, and its quality is a very important nutritional criteria. The uses of plant protein source in aqua feed formulations are of commercial importance to reduce the feed costs (Piedad-Pascual *et al.*, 1990). Since plant protein meals are cheaper than animal protein, it has been focused in substituting the animal sources (Kikuchi, 1999). The antinutritional factors, such as protease (trypsin) inhibitors, phytohaemagglutinin (lectins), anti-vitamins, phytic acid, saponins and phytoestrogens (El-Sayed, 1999; Francis *et al.*, 2001) present in soybean meal can be neutralized while cooking the basal diet. Carbohydrate provides immediate energy followed by protein and lipid, and important for synthesis of chitin, steroids and fatty acids (Clifford and Bricks, 1983). It increases protein sparing effect on growth (Shiau and Peng, 1992). Dietary lipid including cholesterol is also essential to provide essential fatty acids and serves as a source of sterols and phospholipids necessary for growth, maintenance, functional integrity and proper functioning of many physiological processes (Corbin *et al.*, 1983; Kanazawa *et al.*, 1977).

### 3.2. Nutritional Indices in *M. rosenbergii* PL Fed with Ethanolic Extract of *P. amarus* Incorporated Diet

The morphometric data, length and weight, and nutritional indices, such as SR, WG, SGR and PER were found to be significantly increased ($P<0.05$) in 1% of ethanolic extract incorporated feed fed PL followed by 0.5% when compared with control **(Table 2)**. In the case of FCR, the reverse trend was recorded (**Table 2**). The decrease in FCR reflects the better quality of feed offered. Herbal plants and their phytocompounds are used as food ingredients for promoting growth and disease resistance in cultured aquatic animals (Bhavan *et al.*, 2011; Bhavan *et al.*, 2012; Bhavan *et al.*, 2014a; Bhavan *et al.*, 2014b; Citarasu *et al.*, 2003; Direkbusarakom, 2004; Immanuel *et al.*, 2004; Pourmoghim *et al.*, 2015; Rani, 1999; Sivasankar *et al.*, 2015). In the present study, ethanolic extract of *P. amarus* incorporated feed has produced better survival and growth of *M. rosenbergii* PL. The similar effect has been reported in Nutripro-aqua, herbal based diet fed *M. rosenbergii* (Kesavanth *et al.*, 2003). The increase in nutritional indices has also been reported in freshwater prawns fed with *Alteranthera sessilis*, *Allium sativum*, *Cissus quadrangularis*, *Andrographis paniculata*, *Curcuma longa*, *Eclipta alba*, *Trigonella foenum-graecum*, *Zingiber officinale*, *Cynodon dactylon*, *Mentha arvensis*, *Eichhornia crassipes* and *Sargassum cristaefolium* incorporated

feeds (Bhavan *et al.*, 2013a; Bhavan *et al.*, 2013b; Dhanalakshmi *et al.*, 2016; Kanagarasu *et al.*, 2017; Manjula *et al.*, 2018; Poongodi *et al.*, 2012; Radhakrishnan *et al.*, 2013; Shanthi *et al.*, 2012).

**Table-2.** Nutritional indices in *M. rosenbergii* PL fed with ethanolic extract of *P. amarus* incorporated diet

| Parameter | Control | Ethanolic extract of *P. amarus* | |
|---|---|---|---|
| | BI | BI+0.5% | BI+1.0% |
| SR (%) | 73.33±3.33$^{c}$ | 86.66±3.33$^{ab}$ | 90.00±3.33$^{a}$ |
| Length (cm) | 2.80±0.10$^{c}$ | 4.42±0.20$^{ab}$ | 4.86±0.15$^{a}$ |
| Weight (g) | 1.94±0.03$^{c}$ | 2.35±0.03$^{b}$ | 2.58±0.04$^{a}$ |
| LG (g) | 1.10±0.20$^{c}$ | 2.42±0.10$^{b}$ | 2.56±0.25$^{a}$ |
| WG (g) | 1.24±0.02$^{c}$ | 1.31±0.04$^{b}$ | 1.51±0.05$^{a}$ |
| SGR (%) | 0.54±0.03$^{a}$ | 0.34±0.05$^{c}$ | 0.36±0.05$^{b}$ |
| FCR (g) | 0.69±0.03$^{a}$ | 0.66±0.04$^{b}$ | 0.57±0.01$^{c}$ |
| PER (g) | 0.25±0.29$^{c}$ | 3.55±0.73$^{b}$ | 4.09±0.96$^{a}$ |

Each value is mean ± standard deviation of three individual observations.

Initial length and weight were 2.0±0.3cm and 0.91± 0.25g respectively.

Mean values within the same row sharing different alphabetical letter superscripts are statistically significant at $P<0.05$ (one-way ANOVA and subsequent post hoc multiple comparison with DMRT). BI, basal ingredients; SR, survival rate; WG, weight gain, SGR, specific growth rate; FCR, food conversion ratio; PER, protein efficiency ratio

## *3.3. Activities of Digestive Enzymes and Contents of Basic Biochemical Constituents in M. rosenbergii PL Fed with Ethanolic Extract of P. amarus Incorporated Diet*

In addition to food quality, the ethanolic extract of *P. amarus* stimulates secretions of gut in *M. rosenbergii* PL, therefore, activities of protease, amylase and lipase were recorded to be elevated in this study (**Table 3**), which facilitate better digestion and thereby availability of nutrients for absorption and assimilation, which in turn ultimately enhanced the FCR (**Table 2**). The basic biochemical constituents, such as total protein, amino acid, carbohydrate, lipid and ash except moisture, were found to be significantly higher ($P<0.05$) in 1% of ethanolic extract incorporated feed fed PL followed by 0.5% when compared with control (**Table 3**). In the case of moisture content just the reverse was recorded. The food quality, enhanced activities of digestive enzymes and better FCR all led to better survival, growth, and nutritional quality of *M. rosenbergii* PL. The elevations in activities of digestive enzymes and the contents of basic biochemical constituents have also been reported when herbs and herbal extracts incorporated feeds were fed to *M. rosenbergii* PL: *Murraya koenigii* (Direkbusarakom, 2004), *Myristica fragrans and Piper longum* (Bhavan *et al.*, 2013a; Bhavan *et al.*, 2013b) *Syzygium cumini* (Bhavan *et al.*, 2014b), *C. quadrangularis* (Radhakrishnan *et al.*, 2013) *Cynodon dactylon* (Dhanalakshmi *et al.*, 2016), *A. sessilis* (Kanagarasu *et al.*, 2017), *E. crassipes* and *S. cristaefolium* (Manjula *et al.*, 2018) and *Turbinaria ornata* (Rajkumar *et al.*, 2018).

**Table-3.** Activities of digestive enzymes and concentrations of basic biochemical constituents in *M. rosenbergii* PL fed with ethanolic extract of *P. amarus* incorporated diet

| Parameter | | Control | Ethanolic extract of *P. amarus* | |
|---|---|---|---|---|
| | | BI | BI+0.5% | BI+1.0% |
| Digestive Enzymes (U/ mg protein) *(×$10^{2}$ U/ mg protein) | Protease | 0.92±0.02$^{c}$ | 1.13±0.04$^{ab}$ | 1.25±0.07$^{a}$ |
| | Amylase | 0.74±0.06$^{c}$ | 0.82±0.03$^{b}$ | 0.91±0.07$^{a}$ |
| | Lipase* | 0.57±0.07$^{bc}$ | 0.64±0.05$^{b}$ | 0.78±0.03$^{a}$ |
| Basic Biochemical Constituents (mg/ g wet wt.) | Total protein | 83.61±4.31$^{c}$ | 108.13±2.45$^{b}$ | 129.59±3.31$^{a}$ |
| | Total amino acid | 49.01±3.54$^{c}$ | 67.85±1.42$^{b}$ | 81.07±2.95$^{a}$ |
| | Total carbohydrate | 31.34±0.56$^{c}$ | 43.97±1.54$^{ab}$ | 48.19±3.20$^{a}$ |
| | Total lipid | 18.67±2.17$^{c}$ | 24.10±1.58$^{ab}$ | 27.23±1.13$^{a}$ |
| | Moisture (%) | 67.33±1.52$^{a}$ | 57.33±1.15$^{b}$ | 54.66±2.08$^{c}$ |
| | Ash (%) | 13.30±1.12$^{c}$ | 17.00±0.20$^{ab}$ | 18.96±1.70$^{a}$ |

Each value is mean ± standard deviation of three individual observations.

Mean values within the same row sharing different alphabetical letter superscripts are statistically significant at $P<0.05$ (one-way ANOVA and subsequent post hoc multiple comparison with DMRT). BI, Basal ingredients.

The herbal growth promoters enhanced the transcription rate, which in turn led to increased mRNA, total amino acid and finally synthesis of protein (Citarasu, 2010). In the present study, concentration of total protein, amino acid, carbohydrate, lipid and ash were found to be significantly improved in *M. rosenbergii* fed with methnolic extract of *P. amarus* incorporated feed. During growth, accumulation of protein gradually replaces the water content in the muscle tissue (Mayrand *et al.*, 2001). The elevation of protein concentration is directly linked with the translation mechanism occurring within the cells (Thomas *et al.*, 2016). The elevation of nutrient values in *M. rosenbergii* indicates prevalence of the good physiological condition due to *P. amarus*.

## 4. Conclusion

This study concluded that the ethanolic extract of *P. amarus* incorporated diet improves protease, amylase and lipase activities, and total protein, amino acid, carbohydrate, lipid and ash contents in *M. rosenbergii* PL, which led to better survival, growth and nutritional indices. Hence, *P. amarus* can be taken as a feed additive in low cost on-farm feed formulation, which promised for sustainable culture of *Macrobrachium*.

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## Conflicts of Interest

The authors declare no conflicts of interest.

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