International Journal of Research Studies in Biosciences (IJRSB)
Volume 2, Issue 11, December 2014, PP 95-106
ISSN 2349-0357 (Print) & ISSN 2349-0365 (Online)
www.arcjournals.org

Effects of Seeds of Medicinal Plants, *Syzygium Cumini*, *Phylanthus Emblica*, *Azadirachta Indica* and *Ricinus Communis* on Growth Promotion in *Macrobrachium Malcolmsonii* Early Juveniles

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Abstract: *A feeding trial was conducted for a period of 45-days on the freshwater prawn, Macrobrachium malcolmsonii early juveniles fed with seeds of medicinal plants, Syzygium cumini (jambolan), Phylanthus emblica (gooseberry), Azadirachta indica (neem) and Ricinus communis (caster) incorporated feeds. Actually, these medicinal seeds were individually incorporated at three percentage levels (1, 3 and 5%) with basal diet formulated by using fish meal (25%), soya meal (20%), groundnut oil cake (15%), wheat bran (5%), rice bran (10%) and sunflower oil (2%). Tapioca flour (15%) and egg albumin (7%) were used as binding agents. Vitamin B-complex with vitamin-C (1%) was also added. Feed without incorporation of any seed was served as control. These medicinal seeds have acted as appetizer and significantly ($P<0.05$) enhanced the activities of digestive enzymes (protease, amylase and lipase) in S. cumini (5%) incorporated feed fed prawns followed by P. emblica (5%), A. indica (1%) and R. communis (5%) when compared with control. This led to enhanced food consumption, which in turn significantly increases ($P<0.05$) the concentrations of total protein, amino acid, carbohydrate and lipid. Further, the levels of non-enzymatic antioxidants (vitamin-C and E) were also significantly improved ($P<0.05$), which ensures good health of the prawns. Thus, better nutritional indices (survival, growth, and food conversion) were attained. Furthermore, the total haemocyte population was also found to be increased ($P<0.05$), which indicates prevalence of good non-specific immune mechanisms. The activities of enzymatic antioxidants, super oxide dismutase and catalase, and lipid peroxidation were not altered significantly. These states indicate the fact that these medicinal seeds are non-toxic at the tested concentrations. In this study, it was understood that the overall health of M. malcolmsonii was improved due to incorporation of these medicinal seeds. Therefore, these seeds can be taken as supplements in feed formulations for sustainable development of freshwater prawn culture.*

Keywords: *Prawn, Jambolan, Goosberry, Neem, Caster, Survival, Growth*

1. INTRODUCTION

Aquaculture plays a vital role in many countries for offering better nutrition, source of income tool to rural development through better employment opportunities and it also earns foreign exchange. Among the aquaculture species, the edible crustaceans have crucial role in nutritious delicacy for mankind. Freshwater crustaceans, such as *Macrobrachium rosenbergii* and *Macrobrachium mlacolmsonii* are main prawn species used for commercial aquaculture because of their fast growth, attractive size, meat quality and omnivorous feeding habits. There are also good demand for them in domestic and export markets. In 2010 the world production of freshwater prawns are about 5 million tonnes [1]. The monsoon river prawn, *M. malcolmsonii* is widely distributed throughout the Indian subcontinent [2], and it is second largest freshwater prawn after the gain river prawn *M. rosenbergii*. It feeds on decomposing plants and animals, small worms, insects and their larvae. *M. malcolmsonii* has great tolerant to environmental fluctuations and comparatively more resistant to contaminants.

Medicinal plants (traditional herbs) have been widely used in veterinary and human medicine. They are natural products that are not only safe for consumption, but also widely available. In recent years, the herb and herbal products also have a great attention in aquaculture practices. The herbal active principles, such as alkaloids, flavanoids, pigments, phenolics, terpenoids, starch, steroids and essential oils in diet induce the secretion of digestive enzymes, leads to stimulate the appetite, which increases food consumption and food utilization efficiencies, which in turn ultimately promotes growth by inducing transcription and protein synthesis [3-8]. The herbal biomedical practices in aquaculture reduce the side effects that occur due to the application of synthetic compounds, the cost and make it eco-friendly [9]. It have been reported that certain medicinal herbs, such as *Boerhavia diffusa, Solanum nigrum, Terminalia arjuna, Andrographis paniculata, Cissus quadrangularis, Eclipta alba, Withania somnifera, Ocimun sanctum, Alteranthera sessius, Allium sativum, Zingiber officinale, Curcuma longa* and *Trigonella foenum-graecum*, and their commercial products successfully regulated the growth, biochemical and immunological parameters in prawns [3-6, 8, 10].

Syzygium cumini (Myrtaceae), jambolan (Naval Pazham in Tamil) seed is used in various alternative healing systems like Ayurveda to control diabetes, in Unani and Chinese medicine for digestive ailments. Actually, the seed is refuse (about 19%). It has gallic acid and quercetin [11, 12]. It also has secondary metabolites, 7-hydroxycalamenene, methyl-β-orsellinate, β-sitosterol and oleanolic acid [13]. *Phyllanthus emblica* (Phyllanthaceae), the Indian gooseberry (nellikkai), is an important constituent in Ayurvedic medicine. It has antiviral and antimicrobial properties [14]. The seed extract of *P. emblica* contains higher antioxidant activity than fruit extract [15]. *Azadirachta indica* (Meliaceae), Neem (Vembu in Tamil), is considered a major component in Ayurvedic and Unani medicine. Neem seeds are rich in phytoconstituents, which have medicinal and cosmetic applications. N*imbidin* is the main active antibacterial ingredient of neem oil. The neem seed are also described as anthelmintic, antileprotic (cures or prevents leprosy) and antipoisonous. It is an active ingredient in mosquito coils. It has antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative properties. The predominant fatty acids of neem oil are oleic, linoleic, palmitic and stearic acids [16]. *Ricinus communis* (Euphorbiaceae), castor seed (Aamanakku in Tamil) oil contains rich in triglycerides, mainly ricinolein (about 90%), oleate and linoleates are the other significant components. Actually the ricin, is a toxin. Castor oil has tannin, phenol, alkaloid, phytate, oxalate, saponin, cyanogenic glycoside and flavonoid. It also contains various compounds such as cineole, 2- octanol, terpenene-4-ol, limonene, sabinene, pinene, and terpinene [17].

In the view of the active principles of Jambolan seed, Goosberry seed, Neem seed, and Castor seed, the present study was aimed to understand the influence of their incorporation with basal diet on the survival, growth, activities of digestive enzymes (protease, amylase and lipase), concentrations of the basic biochemical constituents (total protein, amino acid, carbohydrate and lipid), contents of non-enzymatic antioxidants (vitamins C and E), activities of enzymatic antioxidants (superoxide dismutase (SOD) and catalase (CAT), quondam of lipid peroxidation (LPO) and total haemocyte population (THC) in the early juveniles of the commercially important freshwater prawn, *M. malcolmsonii*.

2. MATERIALS AND METHODS

2.1. Experimental Animal

The freshwater prawn, *M. malcolmsonii* early juveniles were collected from Anakarai, Kumbakonam District, Tamilnadu, India. They were safely transported to the laboratory in plastic bags half filled with hatchery water and well-oxygenated. They were acclimatized to ambient laboratory conditions for 3 weeks in large cement tank (1000 L) filled with ground water (pH, 7.10±0.40, total dissolved solids, 0.94±0.07 g L^{-1}, dissolved oxygen, 7.20±0.36 mg L^{-1}, BOD, 36.00±1.45 mg L^{-1}, COD, 125.0±7.00 mg L^{-1}, ammonia, 0.018±0.004 mg L^{-1}). Adequate aeration was provided to the prawns.

2.2. Experimental Feeds

The seed of *S. cumini*, *P. emblica*, *A. indica* and *R. communis* were purchased from traditional medicinal shops at Coimbatore, India. These seeds were individually ground to fine powders and stored at room temperature. The branded feed basal ingredients (BI), such as fishmeal (25%),

soybean meal (20%), groundnut oilcake (15%), wheat bran (5%), and rice bran (10%) were taken in powder forms and thoroughly mixed. Sunflower oil (2 %) was used as lipid source. The selected seed powder was individually incorporated with BI in three different concentrations each at 1%, 3% and 5% by replacing the right quantity of BI. Tapioca flour (15%) and egg albumin (7%) were used as binding agents. The dough was steam cooked and cooled at room temperature. Vitamin B-complex forte with vitamin C (1%, BECOSULES® CAPSULES, Pfizer Ltd., Navi Mumbai, India) was also mixed. Sterilized water was adequately added for maintaining the mix in moist and paste form. This mix was pelletized in a manual pelletizer (Kolkata, India) fixed with 3 mm diameter mesh. The pellets were dried in a thermostatic oven (M/s Modern Industrial, Mumbai, India) at 40ºC until they reached constant weight and stored in airtight jars at room temperature. It is important to mention here that freshwater prawn requires 30-40% crude protein, 25-35% carbohydrate and 3-7% lipid [18]. In the present study, the proximate composition of organic matters in the basal diet formulated was determined by adopting the methodology of AOAC [19], which contained more or less 38.5% crude protein, 5.6% crude fat, 7.7% crude fibre, 9.0% total ash, 8.6% moisture and 30.4% carbohydrate (total nitrogen free extract). The water stability of the feeds formulated was checked and the leaching after 8 h of immersion was found to be between 26-30%.

2.3. Feeding Trials

M. malcolmsonii PL (2.28±0.08 cm length and 0.11±0.04 g weight) were starved for 24 h before beginning of the feeding trial. Thirteen groups, each contained 25 prawns were maintained in 25 L plastic tanks in a triplicate experimental set-up. One group served as control and fed with feed formulated by using BI only, and the other groups were fed with experimental feeds prepared by incorporation of each seed (*S. cumini*, *P. emblica*, *A. indica* and *R. communis*) with three different concentrations. The feed was allocated to the prawns for two times a day (8:00 am and 8:00 pm) at 10% of body weight. The experiment was extended for a period of 45 days. The unfed feed, feces and moult (if any) were collected by siphoning method causing minimum disturbance to the prawns on daily basis during renewal of water. A few numbers of feeding trials were conducted then-and-there to meet out the requirement of experimental prawns for analysis of various parameters. For morphometric and nutritional analysis 10 prawns from each group were randomly measured and the mean was considered as a single value (mean of 10 individual measurements = one observation), and three such measurements were made to fulfill the triplicate analysis.

2.4. Nutritional Indices

After the feeding trial, the growth parameters, such as survival rate (SR), length gain (LG), weight gain (WG), specific growth rate (SGR), food conversion rate (FCR) were determined by following equations of Tekinay and Davies [20]. Survival rate, SR (%) = Total No. of live PL / Total No. of prawns introduced initially × 100. Length gain, LG (cm) = Final length (cm) – Initial length (cm). Weight gain, WG (g) = Final weight (g) – Initial weight (g). Specific growth rate, SGR (%) = log w2 – log w1 / t × 100 (where, w1 & w2 represents initial and final weight (g) respectively, and, ‘t’ is the total number of experimental days). Food conversion rate, FCR (g) = Total quantity of feed intake (g) / Weight gain of the prawn (g).

2.5. Digestive Enzymes

The whole flesh except eye stalk and exoskeleton was homogenized in ice cold distilled water and centrifuged at 10,000 rpm under 4ºC for 20 minutes. The supernatant was used as crud enzyme source. The activity of protease was estimated by the method of Furne et al. [21]. One unit of enzyme activity represents the amount of enzyme required to liberate one µg of tyrosine min^{-1} under assay conditions. The activity of amylase was assayed by following the method of Bernfeld [22] in which the increase in reducing power of buffered starch solutions was measured. One unit of amylase activity was calculated as quantity (mg) of maltose liberated/ g of protein/ h (mg/g/h). The activity of lipase was assayed by the method of Furne et al. [21]. One unit of lipase activity was defined as the amount of free fatty acid released from triacyl glycerol per unit time was estimated by the amount of NaOH required to maintain pH constant and represented as mille equivalents of alkali consumed. For these parameters, tissues from five prawns were pooled together from each group to constitute a single observation and three such observations were

made to fulfill the triplicate analysis (5 prawns/ group × 3 parameters = 15 prawns/ group × 3 replicates = 45 prawns).

2.6. Biochemical Constituents

On the initial and final days, the concentrations of basic biochemical constituents, such as total protein, total carbohydrate and total lipid in the muscle of prawns were determined. Concentration of total protein was estimated by the method of Lowry et al. [23] using ethanolic precipitated sample. Concentration of total carbohydrate was estimated by the method of Roe [24] using TCA extracted sample. Concentration of total lipid was extracted by following the method of Folch et al. [25] and estimated by the method of Barnes and Blackstock [26]. For these parameters, tissues from five prawns were pooled together from each group to constitute a single observation and three such observations were made to fulfill the triplicate analysis (5 prawns/ group × 3 parameters = 15 prawns/ group × 3 replicates = 45 prawns).

2.7. Total Haemocyte Count

In a feeding trial at initial and final days of the experiment, 50 µl haemolymph was withdrawn in triplicate from the ventral sinus (in prawn's first abdominal segment) using a 26 gauge hypodermic needle on a 1 ml syringe. The syringe was pre-filled with 150 µl of anticoagulant (10 mM Tris-HCl, 250 mM sucrose, 100 mM sodium citrate, pH-7.6). More anticoagulant was added to make up the volume to 1 ml and the anti coagulated haemolymph was prepared. Further, a volume of 200 µl anti coagulated haemolymph was fixed with an equal volume of formalin (10%) for 30 minutes, and 100 µl of fixed haemolymph was stained with 20 µl of Rose Bengal stain (1.2% Rose Bengal in 50% ethanol) and incubated at room temperature for 20 minutes before being used to determine total haemocyte count (THC). THC was determined by hemocytometer (Neubauer improved, Germany) under the light microscope at RP10x (Labomed, CXR2). THC (cells $\times 10^5$ ml^{-1}) = Counted cells × depth of chamber × dilution factor / Total number of 1 mm square.

2.8. Vitamins

Concentration of ascorbic acid present in TCA extracted tissues sample was measured according to the method of Roe and Kuether [27]. Concentration of α-tocopherol present in petroleum ether-ethanol extracted tissue sample was estimated by the method of Baker et al. [28]. The quantity of vitamin was expressed in µ mol/mg protein.

2.9. Enzymatic Antioxidants and Lipid Peroxidation

The hepatopancreas of test prawns was dissected out and immediately homogenized (10% w/v) in ice-cold 50 mM Tris buffer (pH 7.4), centrifuged at 10,000 g for 20 min at 4ºC and the supernatant was used to assay the enzyme activities. Superoxide dismutase (SOD) activity was measured using pyrogallol (10 mM) autoxidation in Tris buffer (50 mM, pH 7.0) by the method of Kakkar et al. [29]. The specific activity of the enzyme was expressed as units/ mg protein. Catalase (CAT) activity was measured using H_2O_2 as the substrate in phosphate buffer by the method of Sinha [30]. The activity of catalase was expressed as µ moles of hydrogen peroxide consumed/ min/ mg protein.

Lipid peroxidation (LPO) in the tissue homogenates was measured by estimating the formation of thiobarbituric acid reactive substances (TBARS) by following the method of Ohkawa et al. [31]. TBARS was expressed as nmoles of malondialdehyde (MDA)/ mg protein. For these parameters, tissues from five prawns were pooled together from each group to constitute a single observation and three such observations were made to fulfill the triplicate analysis (5 prawns/ group × 3 parameters = 15 prawns/ group × 3 replicates = 45 prawns).

2.10. 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 (v11.5). 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. Growth, Survival and Nutritional Indices

The initial length and weight of *M. malcolmsonii* was 2.28±0.08 cm and 0.11±0.04 g. At the end of the feeding trail, the growth (WG and SGR) and survival were found to be higher in experimental feeds fed prawns when compared with control. In each category, the increase was maximum in *S. cumini* (5%) incorporated feed fed prawns followed by *P. emblica* (5%), *A. indica* (1%) and *R. communis* (5%). These differences were found to be statistically significant (P <0.05). Further, it confirmed by the values of FCR, just the reverse trend of WG and SGR was recorded (Tables 1 and 2). The increases in growth performance have also been previously reported in *M. rosenbergii* fed with following herbal incorporated feeds: *O. sanctum* and *W. somnifera* [3]; *M. koenigii, C. sativum* and *M. arvensis* [4]; *A. paniculata, C. quadrangularis,* and *E. alba* [5]; *A. sessilis, E. alba* and *C. Quadrangularis* [6]; *A. sativum, Z. officinale, C. longa* and *T. foenum-graecum* [8]; *P. longum, P. nigram* and *Z. officinale* [32] and *M. fragrans, G. glabra* and *Q. infectoria* [33].

Table 1. *Morphometric data of M. malcolmsonii early juveniles fed with herbal seeds incorporated feeds*

Feeds		**Length (cm)**	**LG (cm)**	**Weight (g)**	**WG (g)**
Initial		2.28±0.08		0.11±0.04	
Control		3.30±0.18^{d}	1.02±0.13^{f}	0.30±0.07^{d}	0.19±0.02^{c}
S. cumini	1%	3.50±0.08^{c}	1.22±0.07^{e}	0.43±0.02^{c}	0.32±0.03^{b}
	3%	3.76±0.11^{ab}	1.48±0.03^{b}	0.55±0.04^{a}	0.44±0.04^{bc}
	5%	3.94±0.10^{a}	1.66±0.08^{a}	0.56±0.02^{a}	0.45±0.03^{a}
P. emblica	1%	3.60±0.12^{bc}	1.32±0.11^{de}	0.44±0.05^{c}	0.33±0.03^{bc}
	3%	3.74±0.13^{bc}	1.46±0.07^{bc}	0.52±0.06^{ab}	0.41±0.02^{bc}
	5%	3.78±0.13^{ab}	1.50±0.03^{b}	0.55±0.03^{a}	0.44±0.02^{bc}
A. indica	1%	3.76±0.09^{ab}	1.48±0.05^{b}	0.53±0.04^{a}	0.42±0.02^{bc}
	3%	3.62±0.07^{c}	1.34±0.05^{e}	0.45±0.02^{c}	0.34±0.04^{bc}
	5%	3.50±0.11^{bc}	1.22±0.05^{cde}	0.43±0.03^{bc}	0.32±0.02^{bc}
R. communis	1%	3.60±0.07^{bc}	1.32±0.05^{de}	0.44±0.03^{c}	0.33±0.04^{bc}
	3%	3.66±0.11^{bc}	1.38±0.08^{bcd}	0.49±0.04^{abc}	0.38±0.03^{bc}
	5%	3.72±0.06^{b}	1.24±0.06^{bcd}	0.50±0.02^{abc}	0.39±0.02^{bc}

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

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

LG, length gain; WG, weight gain.

Table 2. *Nutritional indices of M. malcolmsonii early juveniles fed with herbal seeds incorporated feeds*

Feeds		**SR (%)**	**SGR (%)**	**FCR (%)**
Control		76.00±8.00^{c}	1.48±0.08^{g}	4.74±0.51^{a}
S. cumini	1%	76.00±8.00^{c}	2.0±0.06^{f}	3.01±0.36^{b}
	3%	88.00±4.00^{ab}	2.31±0.04^{ab}	2.29±0.15^{e}
	5%	92.00±4.00^{a}	2.33±0.09^{a}	2.27±0.22^{e}
P. emblica	1%	80.00±8.00^{bc}	2.02±0.07^{df}	2.80±0.12^{bcde}
	3%	88.00±4.00^{ab}	2.24±0.06^{abc}	2.33±0.18^{de}
	5%	92.00±4.00^{a}	2.31±0.12^{ab}	2.22±0.20^{e}
A. indica	1%	88.00±4.00^{ab}	2.26±0.06^{abc}	2.34±0.32^{de}
	3%	80.00±4.00^{c}	2.04±0.06^{def}	2.90±0.35^{bcd}
	5%	76.00±8.00^{bc}	0.71±0.12^{g}	3.00±0.40^{bc}
R. communis	1%	80.00±4.00^{bc}	2.02±0.05^{df}	2.87±0.12^{bcd}
	3%	80.00±4.00^{bc}	2.15±0.10^{cde}	2.47±0.40^{bcde}
	5%	84.00±4.00^{abc}	2.17±0.09^{bcd}	2.43±0.30^{cde}

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

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

SR, survival rate; SGR, specific growth rate; FCR, food conversion rate.

3.2. Digestive Enzymes

The activities of digestive enzymes, such as protease, amylase and lipase were found to be significantly increased (P <0.05) in experimental feeds fed prawn groups when compared with control (Table 3). Among the three concentrations tested in each seeds, *S. cumini* (5%) incorporated feed fed PL showed the best performance followed by *P. emblica* (5%), *A. indica* (1%) and *R. communis* (5%) when compared with control. Similar increases have been reported in *M. rosenbergii* fed with medicinal herb incorporated feeds: *M. koenigii, C. sativum* and *M. arvensis* [4]; *A. paniculata, C. quadrangularis* and *E. alba* [5]; *A. sessilis, E. alba* and *C. quadrangularis* [6]; *P. longum, P. nigram* and *Z. officinale* [32] and *M. fragrans, G. glabra* and *Q. infectoria* [33].

Table3. *Activities of digestive enzymes in M. malcolmsonii early juveniles fed with herbal seeds incorporated feeds*

Feeds		**Protease (U/mg protein)**	**Amylase (U/mg protein)**	**Lipase (U/mg protein ×10^3)**
Initial		0.26±0.03	0.15±0.04	0.73±0.07
Control		0.76±0.07^{g}	0.28±0.09^{f}	0.82±0.11^{g}
S. cumini	1%	0.82±0.08^{fg}	0.31±0.05^{ef}	0.89±0.06^{fg}
	3%	1.23±0.19^{de}	0.58±0.10^{abc}	1.26±0.15^{abc}
	5%	1.87±0.26^{a}	0.65±0.07^{a}	1.36±0.09^{a}
P. emblica	1%	1.06±0.09^{def}	0.37±0.11^{def}	0.94±0.09^{efg}
	3%	1.34±0.16^{cd}	0.55±0.073^{abcd}	1.17±0.1^{bcd}
	5%	1.72±0.21^{ab}	0.61±0.14^{ab}	1.32±0.08^{ab}
A. indica	1%	1.56±0.17^{bc}	0.56±0.09^{abc}	1.21±0.07^{abc}
	3%	1.17±0.10^{efg}	0.45±0.06^{bcdef}	1.02±0.06^{dfe}
	5%	0.94±0.13^{de}	0.34±0.13^{ef}	0.93±0.04^{efg}
R. communis	1%	1.13±0.15^{de}	0.40±0.08^{cdef}	0.97±0.05^{efg}
	3%	1.22±0.21^{de}	0.47±0.13^{abcde}	1.08±0.12^{cde}
	5%	1.29±0.18^{cd}	0.53±0.11^{abcd}	1.15±0.14^{bcd}

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

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

3.3. Biochemical Constituents, Vitamins, Minerals and Population of Hemocytes

The basic biochemical constituents, such as total protein, carbohydrate and lipid, non-enzymatic antioxidants, such as vitamin-C and E, and total hemocytes population were found to be significantly increased (P <0.05) in experimental feeds fed PL groups when compared with control (Tables 4 and 5). Among the three concentrations tested in each seed, *S. cumini* (5%) incorporated feed fed prawns showed the best performance followed by *P. emblica* (5%), *A. indica* (1%) and *R. communis* (5%). The increase in concentrations of biochemical constituents have also previously been reported in *M. rosenbergii* fed with herb incorporated feeds: *O. sanctum* and *W. somnifera* [3]; *A. paniculata, C. quadrangularis* and *E. alba* [5]; *A. sativum, Z. officinale, C. longa* and *T. foenum-graecum* [8]; *M. koenigii, C. sativum* and *M. arvensis* [4]; *A. sessilis, E. alba* and *C. quadrangularis* [6]; *P. longum, P. nigram* and *Z. officinale* [32] and *M. fragrans, G. glabra* and *Q. infectoria* [33].

Table 4. *Contents of biochemical constituents in M. malcolmsonii early juveniles fed with herbal seeds incorporated feeds*

Feeds		**Protein (mg/g)**	**Carbohydrate (mg/g)**	**Lipid (mg/g)**
Initial		75.73±3.48	19.05±2.76	4.56±1.16
Control		124.25±4.07^{g}	28.00±2.37^{i}	9.42±2.07^{d}
S. cumini	1%	149.47±6.12^{de}	31.21±2.45^{hi}	11.26±2.08^{cd}
	3%	192.22±5.96^{b}	51.23±4.12^{c}	16.41±2.73^{abc}

	5%	214.28±4.49^{a}	64.62±4.57^{a}	19.79±3.12^{a}
P. emblica	1%	138.44±4.18^{f}	36.47±3.48^{gh}	12.25±2.76^{cd}
	3%	196.84±5.24^{b}	47.18±2.73^{cd}	14.30±3.05^{bcd}
	5%	212.81±3.47^{a}	58.06±3.15^{b}	17.84±2.18^{ab}
A. indica	1%	207.52±3.92^{a}	48.59±4.28^{cd}	15.53±3.51^{abc}
	3%	174.46±5.71^{c}	40.14±2.76^{efg}	13.53±2.17^{bcd}
	5%	137.27±4.54^{f}	34.14±2.83^{gh}	11.68±2.64^{cd}
R. communis	1%	142.86±5.19^{ef}	37.83±4.51^{fg}	12.37±2.43^{cd}
	3%	152.81±4.35^{d}	42.93±2.94^{def}	13.74±2.61^{bcd}
	5%	173.18±5.08^{c}	44.75±3.69^{de}	13.89±2.90^{bcd}

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

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

Table 5. *Concentrations of vitamin-C and E, and total haemocytes count in M. malcolmsonii early juveniles fed with herbal seeds incorporated feeds*

Feeds		**Vitamin – C (µmol/mg protein)**	**Vitamin - E (µmol/mg protein)**	**THC (cells x10^{5} ml^{-1})**
Initial		17.28±2.78	8.20±1.27	45.67±3.14
Control		43.66±2.54^{g}	20.90±2.12^{f}	55.75±4.10^{g}
S. cumini	1%	50.17±2.50^{f}	23.61±2.84^{ef}	63.45±3.75^{f}
	3%	66.61±3.71^{ab}	35.26±3.35^{ab}	78.67±3.73^{abc}
	5%	69.58±3.52^{a}	37.25±3.02^{a}	81.46±3.65^{a}
P. emblica	1%	55.94±2.97^{de}	24.72±1.93^{def}	69.41±4.18^{def}
	3%	63.95±2.56^{abc}	32.55±3.07^{abc}	76.67±3.73^{abc}
	5%	67.73±3.83^{ab}	36.14±2.94^{a}	79.88±3.65^{ab}
A. indica	1%	64.26±2.94^{abc}	33.71±2.81^{ab}	77.88±3.65^{abc}
	3%	59.36±3.21^{cd}	27.59±3.52^{cde}	72.23±4.60^{bcd}
	5%	53.68±2.73^{ef}	24.05±2.79^{ef}	65.38±4.38^{ef}
R. communis	1%	56.18±2.87^{de}	25.67±3.26^{def}	71.48±4.13^{cde}
	3%	62.52±3.09^{bc}	28.32±2.57^{cde}	74.45±3.75^{abcd}
	5%	63.47±3.57^{bc}	30.07±3.12^{bcd}	75.34±3.06^{abcd}

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

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

THC, Total haemocyte count

Dietary protein utilization is mainly controlled by its amino acid composition, calorie content, digestibility, physiological state and size of the individual, and water temperature [34]. Certain herbs, *Massa medicata, Cratae gifructus, Artemisia capillaries* and *Cnidium officinale* promoted cellular lipid and fatty acid utilization, which facilitates protein accumulation and good growth in *Pagrus major* [35]. *W. somnifera* fed spawners showed higher protein values in the haemolymph that positively regulated the larval quality in *P. monodon* [36]. It has been reported that the herbal growth promoters helped to induce the transcription, leading to increased RNA, which coupled with increased amino acid and finally enhanced protein synthesis [7].

The increased contents of vitamin-C and E indicated the fact that the general health of test prawns was improved due to incorporation of these medicinal seeds. Ascorbic acid is a potent antioxidant which scavenges reactive free radicals, such as hydroxyl, perhydroxyl, peroxyl and nitric oxide [37, 38, 39]. Ascorbic acid is believed to regenerate vitamin E from its oxidized form, thereby raises the antioxidant status [40].

The increase in haemocytes population has also been reported in *M. rosenbergii* fed with *A. sessilis*, *E. alba* and *C. quadrangularis* [6]. The haemocytes population is used as an indicator of crustacean health status [41]. In *M. rosenbergii*, incorporation of anthraquinone from *Rheum officinale* improved activities of haemolymph lysozyme, alkaline phosphatase and antioxidation abilities, leads to improved growth [42]. In grouper (*Epinephelus tauvina*), herbs, *O. sanctum*, *W. somnifera* and *M. fragrans* have improved the immune parameters, such as phagocytic activity, serum bactericidal activity, albumin-globulin ratio and leukocrit against *Vibrio harveyi* [43]. In

goldfish (*Carassius auratus*), herbs, *Emblica officinalis*, *Cyanodon dactylon* and *Adhatoda vasica* have increased the leucocyte number [44]. In *E. tauvina*, extracts of *Viscum album*, *Urtica dioica* and *Z. officinalis* improved the non-specific defense mechanisms, including extracellular and intracellular respiratory burst activities, phagocytosis in blood leukocytes, total plasma protein, specific growth rate and condition factor [45]. Therefore, in the present study, the increased hemocytes population indicates prevalence of good non-specific defense mechanisms, and thus, *P. somniferum*, *E. cardamomum*, *F. vulgare*, and *R. communis* seeds have immune stimulant property.

3.4. Antioxidant Enzymes and Lipid Peroxidation

Activities of enzymatic antioxidants, SOD and CAT were not altered significantly between control and *S. cumini*, *P. emblica*, *A. indica* and *R. communis* seed incorporated experimental feeds fed prawn groups (Table 6). These results reflect the fact that these seeds are non-toxic at the tested concentrations. However, in the case of *A. indica*, activities of SOD and CAT were found to be elevated in 3% and 5% incorporations (Table 6). The process of LPO in *S. cumini*, *P. emblica*, *A. indica* and *R. communis* seed incorporated experimental feeds fed prawn was decreased when compared with control. Maximum decrease was seen in, 5% *P. emblica* followed by, 5% *R. communis*, and 5% *S. cumini* (Table 6). However, an elevation in LPO was observed in 3% and 5% of *A. indica* incorporation (Table 6). This indicates the fact that the free radicals generation was increased when the concentrations of *A. indica* seed was increased beyond optimized level.

Table 6. *Activities of enzymatic antioxidants (SOD and CAT) and lipid peroxidation in M. malcolmsonii early juveniles fed with herbal seeds incorporated feeds*

Feeds		**SOD (µmol H_2O_2consumed/ min/mg protein)**	**CAT (U/mg protein)**	**LPO (nmol MDA/ mg protein)**
Initial		20.47±1.74	13.78±1.58	0.78±0.13
Control		$58.32±4.09^{ab}$	$28.42±2.36^{b}$	$2.77±0.38^{f}$
S. cumini	1%	$60.18±2.84^{ab}$	$29.06±1.83^{b}$	$2.70±0.16^{bc}$
	3%	$59.13±2.39^{ab}$	$28.30±1.28^{b}$	$2.56±0.13^{bc}$
	5%	$58.03±1.80^{ab}$	$28.55±1.31^{b}$	$2.48±0.15^{cd}$
P. emblica	1%	$56.61±3.84^{ab}$	$29.55±2.17^{b}$	$2.45±0.15^{cd}$
	3%	$55.73±2.38^{b}$	$29.63±2.61^{b}$	$2.33±0.17^{d}$
	5%	$57.91±3.19^{ab}$	$28.03±1.71^{b}$	$1.98±0.22^{e}$
A. indica	1%	$57.93±2.49^{ab}$	$28.65±2.18^{b}$	$2.63±0.11^{bcd}$
	3%	$60.21±3.51^{ab}$	$30.41±2.75^{b}$	$2.99±0.12^{ab}$
	5%	$62.75±3.47^{a}$	$34.97±3.23^{a}$	$3.28±0.13^{a}$
R. communis	1%	$59.50±4.25^{ab}$	$29.01±2.05^{b}$	$2.68±0.18^{bcd}$
	3%	$59.00±3.97^{ab}$	$28.58±1.53^{b}$	$2.40±0.18^{cd}$
	5%	$58.00±2.05^{ab}$	$28.41±1.94^{b}$	$2.37±0.22^{cd}$

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

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

SOD, superoxide dismutase; CAT, catalase; LPO, lipid peroxidation; MDA, melandialdehyde.

Reactive oxygen species (ROS), include hydroxyl radical ($^{\bullet}HO$), such as the neutral form of the hydroxide ion (HO^{-}), superoxide (hyperoxide) anion (O_2^{-}), hydrogen peroxide (H_2O_2), and singlet oxygen (O_2*, dioxidene and dioxygen) are physiologically generated through a series of biochemical reactions within cellular compartments and increase in physiological conditions that result in oxidative stress, disease and immune defense reactions [46]. The increase in ROS production would leads to irreversible cellular damage and cell death. In normal cells, there exist a delicate balance between the pro oxidant forces and antioxidant defences known as redox balance. Nevertheless, over whelming of antioxidant defences of cells by pro oxidants leads to oxidative stress. It is reported that, oxidative stress in aquatic organisms is more profound during nutritional deficiency, elevated

temperature, hypoxia and exposure to xenobiotics [47]. However, either an increase in ROS production above the level that can be removed by antioxidant defences or a decrease in the capacity of the antioxidant defences could result in oxidative damage to key molecules, including DNA, protein and lipids [38]. In such a condition, the oxidative degradation of lipids (LPO) would be occurred. SOD plays a crucial role in the defense against oxidative cellular damage, through catalyzing the breakdown of O_2^- to O_2 and H_2O_2 [48]. CAT catalyzes the decomposition of H_2O_2 to water and oxygen. In the present study, since no predominant lipid peroxidation occurred, there may be less ROS production, and thus insignificant changes in activities of SOD and CAT were recorded in all levels of supplementations of *S. cumini*, *P. emblica* and *R. communis*, and at 1% level of supplementation of *A. indica*.

In this study, seeds of *S. cumini*, *P. emblica*, *A. indica* and *R. communis* induced secretion of digestive enzymes in *M. malcolmsonii* early juveniles, which suggest increased food consumption and absorption of nutrients and therefore, elevation of basic biochemical constituents including total protein and vitamins were resulted. Further, the lowered lipid peroxidation suggests less ROS production, and therefore, insignificant changes in activities of SOD and CAT were recorded, which ultimately indicates the fact that these herbal seeds were non-toxic to *M. malcolmsonii* early juveniles at tested concentrations. Furthermore, the elevated hemocytes population ensures prevalence of immune modulation and good non-specific defence mechanism, which favours good health, which in turn ultimately resulted in better growth and survival of *M. malcolmsonii* early juveniles. Therefore, these herbal plant seeds can be incorporated in aqua feed formulations for sustainable development of *Macrobrachium* culture.

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