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EFFECT OF *SARGASSUM* POWDER ON GROWTH AND REPRODUCTIVE POTENTIALS OF BRINE SHRIMP *ARTEMIA PARTHENOGENETICA*

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ABSTRACT

Dietary influence of *Sargassum* powder (250mg, 500mg, 750mg and 1000mg) on the growth and reproductive capabilities of *Artemia parthenogenetica* fed with rice bran has been described in this paper. At the end of 5th week, the survival percentage was increased from 51.6±2.46% to 59.0±1.51%. The maximum life span of *Artemia* was extended from 90.6±2.09 (control) to 94.3±2.93 days. The length of *Artemia* increased from 83.2±1.61mm (control) to 85.3±2.41mm. The biomass of *Artemia* increased from 76.3±1.62 mg (control) to 84.9±1.17mg. The FCR increased from 69.2±1.68% (control) to 71.4±1.74%. The maturation period decreased from 23±2.58 days (control) to 15±2.58 days. Gestation period decreased from 5.8±1.09 days (control) to 4.8±0.81 days. Reproductive period was extended from 32.3±3.30 days (control) to 37.1±3.79 days. *Sargassum* powder increased the number of broods/*Artemia* from 5.03±0.61 (control) to 7.3±0.59 while the inter-brood duration was reduced from 4.62±0.48 days (control) to 4.26±0.56. The number of nauplii per brood increased from 43.26±2.21to 59.38±2.09, but the number of cysts/ brood decreased from 1.2±0.19 to 0.8±0.1. The diameter of cyst reduced from 241.4±29.4µm to 227.3±18.2µm (at 500mg). Hatching percentage of cysts increased significantly ($p<0.05$) from 54.2±2.07% (control) to 68.2±2.56% (at 500mg). Fecundity of *Sargassum* fed cultures of *Artemia* was 251.4±9.43 instead of 181.04±7.68 in the control. The post reproductive period in the *Sargassum* fed *Artemia* was 11.4±1.19 days instead of 10.3±1.39 in the control. *Sargassum* powder provided some extra amounts of crude proteins, dietary fibres, essential amino acids, fatty acids, minerals and vitamins in addition to the normal amounts supplied by rice bran. Hence, *Sargassum* powder supports the growth and biomass production as well as the reproductive attributes of *Artemia*, but higher doses do not have such effects due to excess of minerals. The maximum dietary supplementation effect was noted at 500mg dose of *Sargassum*.

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INTRODUCTION

Artemia is a live feed to most aquaculture animals which need high quality energy feeds at least for certain stage of their development. Intensive *Artemia* culture has been the centre of interest to maximize the biomass and cyst production either by providing adequate amount of feeds rich in valuable substances or by enriching the *Artemia* by providing certain feed supplements, plant extracts or drugs. Efficiency of these feeds in promoting growth and reproductive potentials of *Artemia* has been investigated in conical flask cultures under standard laboratory conditions (Dana and Lenze, 1986) and the most effective feed has been used in the intensive cultures via batch culture, semi-through culture, flow- through culture (Thobias *et al*., 1985) and high density flow through culture (Brisset *et al*., 1982; Levens *et al*., 1985). As in natural habitat, the seawater medium is best and of economic interest to grow *Artemia* in concrete tanks (Dana and Lenze, 1986). For enhancing the growth and reproductive performances of *Artemia*, several attempts have been taken to evaluate suitable feeds that enable it to produce more biomass and cysts to meet the market demand. Thobias *et al*. (1980) fed *A. franciscana* and *A. parthenogenetica* with *Chaetoceros curvestus* (Bacillariophyceae) under laboratory conditions and compared

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their growth and productivity with those of other strains. Vanhaecke *et al*. (1984) cultured *A. parthenogenetica* using *Dunaliella fertiolata* as feed. Yashiro (1985) studied the growth and survival of *A. salina* in seawater medium fed with powdered dry organic manures such as rice bran, chicken manure, cow dung and pig manure. Lavens *et al*. (1986) reared *A. franciscana* and *A. parthenogenetica* by providing *Spirulina* as feed component. Wear and Haslett (1986) supplied *Dunaliella euchlora* and *D. salina* as feeds to *A. franciscana*. Gozalbo *et al*. (1987) cultured *A. franciscana* and *A. parthenogenetica* using feeds such as *Asteromonas*, *Chlorella*, *D. bardavill, Nanaochloropsis*, *Phaeodactylum tricormutum* and *Synechococcus*, and analyzed their growth and biochemical compositions in relation to the feed types. Balasundaram *et al*. (1987) grew *A. parthenogenetica* in the seawater medium containing *Spirulina* as feed. Vijayaraghavan *et al*. (1987) grew *A. parthenogenetica* using feeds like *Enteromorpha intestinalis, Porphyra vietnamensis, Ulva* (green algae), *Isochrysis glabra* and rice bran. Lavens *et al*. (1987) evaluated mono and mixed diets for *A. franciscana* using corn flour, soybean flour and rice bran. Berthelemy – Okazaki *et al*. (1987) grew *A. franciscana* using *Tetraselmis* (Bacillariophyceae) and studied the impacts of environmental factors on the growth, reproduction and cyst formation. Castelo Branco *et al*. (1987) investigated the larval growth of *A. parthenogenetica* by feeding it with dried microalgae such as *Chlorella* and *Scenedesmus*. Ronsivalli *et al*. (1987) fed *A. franciscana* using rice bran and whey powder while Bargava *et al*. (1987) grew *A. parthenogenetica* using pig dung and groundnut oil cake. Browne *et al*. (1988) grew *A. franciscana* and *A. parthenogenetica* by providing yeast and *Dunalella* as feeds. Basil *et al*. (1989) cultured *A. parthenogenetica* in an open system fed with rice bran, groundnut oil cake, coconut husk, cabbage leaves, soybean flour, tapioca flour and cattle blood in different composition.

Marian *et al*. (1991) grew *A. parthenogenetica* using organic wastes supplemented with rice bran; meanwhile Kitto *et al*. (1991) cultured *A. franciscana* using *Chlorella salina*. Basil and Pandian (1991) reared *Artemia* using groundnut oil cake, decayed cabbage leaves, super phosphate, baker's yeast, poultry litter and biogas slurry in different composition as feeds. Basil *et al*. (1991) used *Enteromorpha compressa* as feed to *A. franciscana*. Nimura *et al*. (1994) fed *A. franciscana* with a marine species of *Chlamydomonas*. Barata *et al*. (1995) grew *A. tunisiana* and *A. parthenogenetica* in the artificial seawater medium fed with *Dunaliella*. Basil *et al*. (1995) grew *A. franciscana* and *A. parthenogenetica* by feeding them with cow dung, cabbage leaves, poultry manure and pig manure.
Indira (1996) prepared Lactobacilli enriched fed to grow *A. parthenogenetica*. Maheswari (1996) grew *A. parthenogenetica* using rice bran feed supplemented with different proportions of baker's yeast. Yoganandhan and Sahul Hameed (2000) evaluated the powdered husks of red gram and black gram as feeds for brine shrimp. Chau Minh Khoi *et al*. (2006) used *Chaetoceros calcitrans* as feed to *A. franciscana* in ponds. Of these feeds tested under laboratory conditions, rice bran seems to be best and economic for conical flask cultures. The brown seaweed *S. wightii* is a macroscopic marine alga found attached to rocky bottom of shallow coastal waters of Tamilnadu and many parts of Asia, which has been used as animal feed, food ingredients, fertilizer, medicines and raw materials (Levering *et al*., 1969). This marine alga is a good source of minerals, vitamins (A, B_1, B_2, C, D and E), proteins, essential amino acids, fats, fatty acids, polysaccharides, fibers and flavanoids (Lahaye, 1991 and Darcy-Vrillon, 1993) and its protein and lipid qualities are acceptable for humans and veterinary animals as it contains relatively high proportion of essential amino acids and unsaturated fatty acids (Thillaikkanu Thinakaran *et al*., 2012). Dietary supplementation of *S. wightii* has enhanced the weight gain of mice (Yung-Choon Yoo *et al*., 2007), tiger shrimp (Felix *et al*., 2004.2005), ducks (Breikaa, 1993) and poultry (El-Deek *et al*. 2011). As this algal powder is rich in acceptable proteins, it has been recommended as a dietary supplement for cattle in China, Thailand, Korea, Japan, Indonesia and Philippines (Kolanjinathan *et al*., 2014). The present study was undertaken to analyze the suitability of the dried powder of *S. wightii* for using as a feed to *Artemia parthenogenetica*.

MATERIALS AND METHODS

Materials

For this *in vitro* culture, the starting materials called cysts of *A. parthenogenetica* were obtained from the salt pans of Tuticorin located on 8º 47' Northern latitude and 77º 68' Eastern longitude, and used in this study. Rice bran was purchased from local markets in Nagercoil and used as a feed to *Artemia*. This feed contained 3.40% moisture, 7.0% protein, 1.59% lipids, 53.06% carbohydrate and 5.91% free amino acids in its dry weight and is in convenient size that can be consumed by both nauplii and adults of *Artemia*. Seawater was collected from the Indian Ocean near Sanguthurai beach of Kanyakumari district and used as medium for the laboratory culture of the *Artemia* strain.

The physico-chemical parameters of the seawater are given hereunder

Salinity -32.3ppm
pH - 8.13
Light penetration - 0.9m
Dissolved oxygen - 2.65ml/L
Temperature - 28.5 ºC (day time)
- 23.2 ºC (night time)
Total phosphorus - 2.37µgm/L
Nitrates - 6.43 µgm/L
Nitrite - 0.62 µgm/L
Primary productivity -154mg c/m^3/hr.

Methods

Preparation of *Sargassum* powder

Specimens of *S. wightii* (Phaeophyceae) were collected from the coastal village Leepuram near Kanyakumari (Lat 9º11′ N; Long79º 24′E) of Tamilnadu and brought to the laboratory. They were washed repeatedly with tap water for 3 times to remove dust particles, sand and epiphytic microalgae. The whole plants were dried under shade, and then sun dried and ground into *Sargassum* powder. This powder was then stored in a refrigerator.

Chemical analysis of *Sargassum* powder and Rice bran

The protein content of *Sargassum* powder was estimated using the Biurrette method described by Raymont *et al.* (1964). The lipid was extracted using chloroform methanol mixture as a

solvent and estimated by using the method described by Folch *et al.* (1956). The method described in the AOAC (1995) was followed for the quantification of minerals in *Sargassum* powder. A 0.2 g of oven dried *Sargassum* powder was taken in a dry conical flask and treated with 10 ml of diacid mixture (2:5 of Nitric acid and Perchloric acid). The content of conical flask was allowed to stand for a few hours for cold digestion. After that, the conical flask was kept on a hot plate to digest the contents under the influence of temperature. The digested content was filtered through a Whatman No.40 filter paper to get a filtrate. The filtrate was suitably diluted and fed into ICP - Perkin Elmer Mayer Optical Emission Spectrophotometer (Optima 2100 DV) as per the procedure given in the Users' Manual for analyzing the amount of Mg, Cu, Mn, Fe and Zn present in the filtrate. Na, K, I and Ca were analyzed with Flame Photometer. For the estimation of methionine, cystine and lysine contents, one gram of *Sargassum* powder was hydrolyzed with 6N hydrochloric acid in evacuated sealed tube for 24 hours at 110ºC and the hydrolyzed sample was analyzed with Waters Pico-Tag HPLC Amino acid Analysis System (Column: Pico-Tag amino acid analysis column, 3.9 (150 mm); detector: Waters 2489 Dual λ absorbance detector). The same methods were used for the rice bran also.

Preparation of Nauplii

The cysts of *A. parthenogenetica* were decapsulated chemically by chlorine bleach treatment described by Granvil D Treece (2000). About 1gram of cysts was incubated in 15ml of freshwater at room temperature for 1hour and the hydrated cysts were concentrated and poured into a chilled decapsulation solution containing 10ml of chlorine bleach (NaOCl; 5.5%) and 5ml of sodium hydroxide (NaOH; 40%) and incubated at 10ºC for 5 minutes. After decapsulation, 3.5g of sodium thiosulfate ($Na_2S_2O_3$) was added to the decapsulation vessel to neutralize the chlorine. A 250ml of seawater was taken in a conical flask and its pH was adjusted to 8.0 by adding 1.0% sodium bicarbonate solution; the flask was gently warmed to 28ºC±1ºC to assist in the hatching process. About 500mg of decapsulated *Artemia* cysts was added into the flask containing seawater and the flask was exposed to 1000 Lux light per day using a fluorescent tube light. Continuous aeration was given to the bottom of the flask at the rate of 10 to 20 lit. per minute. After 24 hrs of incubation, young nauplii were collected by scooping the top of the medium using a 100µm harvesting mesh bag as done by Lavens and Sorgeloos (1987). The instar I nauplii in the medium were counted by diluting 1ml of sample with 99 ml of seawater so as to have 1000 instars/ 1ml of medium.

Culture of *Artemia*

A 1.5 lit of seawater was taken in a large glass vessel and its pH was adjusted to 8.0 by adding 1.0% sodium bicarbonate solution and slightly warmed to 28ºC±1ºC to provide optimum growth temperature. Triplicates of 5 bowls of 100ml capacity were cleaned well, filled with 100ml of the seawater in each bowl. 1 ml of medium containing about 1000 instar I nauplii was inoculated in each of the bowls which were then kept on a clear table in a room fitted with a fluorescent tube light capable of illuminating 1000 Lux light. Continuous aeration was given to the bottom of the bowls at the rate of 10-20 lit per minute to maintain proper level of dissolved oxygen in the medium. A 100gm of rice bran was dissolved in 200ml of water to get a feed suspension and 1ml of that feed solution was provided to the first bowl (control) daily, once in the morning and evening. A 100gram of Sargassum powder was dissolved in 200ml of water and the bowls were supplied with

The following feeds both in the morning and evening

Bowl I -500mg rice bran (1ml feed solution)
Bowl II -Rice bran +125mg Sargassum powder
Bowl III -Rice bran +250mg Sargassum powder
Bowl IV -Rice bran +375mg Sargassum powder
Bowl V -Rice bran +500mg Sargassum powder

Measurement of Growth Characteristics

To determine the survival rate, the number of *Artemia* in each and every treatment was individually counted regularly once in five days intervals up to the death of last individual and the survival rate was calculated from the data by using the formula:

$$\text{Survival rate (\%)} = \frac{\text{Density of } \textit{Artemia} \text{ on the day of counting}}{\text{Density of } \textit{Artemia} \text{ on the first day of culture}} \times 100$$

where, density of *Artemia* denotes the number of *Artemia* per ml of culture. The average life span was calculated by dividing the cumulative percentage survival with the maximum survival in days.

$$\text{Average life span (days)} = \frac{\text{Cumulative Survival (\%)}}{\text{Maximum Survival (day)}}$$

Growth of Artemia was measured in terms of length from the tip of head to the base of caudal furca using a hand lens and graph paper. A few *Artemia* along with a drop of water were transferred onto a glass slide, that slide was placed over the graph paper and the length of the animal was read against the lines of the graph paper. The length of 20 individuals from each sample was measured to determine the mean length (mm) and standard deviation as the growth index. *Artemia* biomass was estimated on the 30th day of the cultures. 10ml sample of a culture was pipetted out and filtered through a dry Whatmann No.1 filter paper to remove extraneous water from the *Artemia* as a measure to estimate the exact biomass by wet weight analysis using a monopan balance (OHD-160, DHONA company, India). Food conversion efficiency of *Artemia* was estimated by dividing the total dry weight of rice bran added to the culture up to 30th day by wet weight of *Artemia* on that day.

$$\text{Food Conversion efficiency (gm)} = \frac{\text{Wet weight of Artemia}}{\text{Total dry weight of rice bran}} \times 100$$

Estimation of Reproductive Characteristics

To estimate the maturation period, a few drops of culture were taken daily and placed on a glass slide and then observed under a dissecting microscope to visualize the brood pouch on either side of the abdomen. The duration taken for the development of brood pouch was considered to be the maturation period. Gestation period is the duration between the development of brood pouch in 5% of individuals and the release of first brood. The new brood was known by observing nauplii in the liquid sucked in a pipette. The reproductive period, number of broods released by a female, number of

nauplii and cysts produced per day and duration between broods were also estimated from the subculture experiments. This method was also used to determine the post reproductive period of *Artemia*, the time interval between last brood parturition and death of the animal. The cyst diameter was measured with the aid of an ocular micrometer as discussed by Aneja (1993). The percentage of females in the total number of individuals was estimated by observing the brood pouches. Fecundity of *Artemia* was determined from the total number of nauplii and/or cysts produced by a female and survival percentage on the 15th day on which most *Artemia* became adults capable of reproduction. It was calculated from the data using the formula-

$$\text{Fecundity} = \frac{\text{Number of nauplii and/or cysts produced by a female}}{\text{Survival percentage on 15}^{\text{th}}\text{ day of the culture}} \times 100$$

where, the number of nauplii produced by a female was calculated by multiplying the number of nauplii per brood with number of brood per female.

RESULTS

Proximate Concentration of Nutrients

Chemical analysis shows that 1 gram of rice bran (basic feed) contains crude proteins (8.9%), crude fats (2.6%), dietary fibres (12.5%), essential amino acids such as cystine (7.9mg), histidine (4.1mg), leucine (10.4mg), isoleucine (6.4mg), methionine (2.8mg), phenylalanine (5.7mg), tryptophan (2.8mg), tyrosine (5.4mg), and valaine (8,2mg), minerals like calcium (1.0mg), phosphate (16mg), chloride (0.7mg), sodium (0.7mg), manganese (0.124mg), zinc (0.057mg), potassium (0.017mg), saturated fatty acids (7.4mg), monounsaturated fatty acids (6.6mg) and polyunsaturated fatty acids (9.5mg). Likewise, 1 gram of Sargassum powder contains crude proteins (12.3%), crude fats (3.4%), dietary fibres (32.2%), essential amino acids such as cystine (2.5mg), histidine (4.5mg), leucine (5.5mg), isoleucine (5.6mg), methionine (5.2mg), phenylalanine (3.7mg), tryptophan (6.4mg), tyrosine (5.3mg), and valaine (5.0 mg), minerals like calcium (67.2mg), phosphate (45.6mg), chloride (23.2mg), sodium (45.1mg), manganese (5.38mg), zinc (1.81mg), potassium (61.3mg),

Table 1. Proximate concentration of various nutritional components in the experimental feeds

Chemical components	Rice bran (1000 mg)	Addition of Sargassum powder/day			
		250 mg	500mg	750 mg	1000mg
Crude protein %	8.9	11.6	14.6	17.7	20.8
Crude lipids %	2.6	3.5	4.3	5.2	6.0
Dietary fibres %	12.5	20.6	29.1	37.6	46.1
Amino acids					
Cystine (mg)	7.9	8.5	9.3	9.9	10.5
Histidine (mg)	4.1	5.2	6.3	7.4	8.5
Leucine (mg)	10.4	11.7	13.1	14.4	15.6
Isoleucine (mg)	6.4	7.8	9.2	10.6	11.0
Lycine (mg)	7.8	9.1	10.4	11.7	13.1
Methionine (mg)	2.8	3.7	4.5	5.3	6.2
Phenylalanine	5.7	7.3	8.9	10.5	11.9
Tryptophan (mg)	2.8	3.5	4.1	4.7	5.3
Tyrosine (mg)	5.4	6.7	7.0	8.1	9.2
Valine (mg)	8.2	9.5	11.3	12.7	13.8
Minerals					
Calcium (mg)	1.0	17.8	41.8	58.6	77.2
Phosphate (mg)	16	27.4	41.6	57.4	71.0
Chloride (mg)	0.7	6.5	12.3	18.2	23.9
Sodium (mg)	0.7	11.9	23.2	34.8	35.8
Manganese (mg)	0.124	1.7	2.9	4.4	5.6
Zinc (mg)	0.057	0.51	1.12	1.67	2.97
Potassium (mg)	0.017	15.8	31.6	46.2	63.3
Fatty acids					
Saturated fatty acids (mg)	7.4	10.3	13.2	15.4	16.6
Monounsaturated fatty acids (mg)	6.6	8.9	11.2	13.4	15.6
Polyunsaturated fatty acids (mg)	9.5	12.0	15.1	18.3	21.5

Table 2. Changes in the reproductive characteristics of Artemia parthenogenetica in response to different doses of Sargassum diet

Characteristics	Rice bran (1000 mg /day)	Weight of Sargassum feed /day			
		250 mg	500mg	750 mg	1000mg
Maturation period (day)	23±2.58[a]	17.8±0.83[b]	16.4±2.14[b]	16.0±1.22[b]	15±2.58[b]
Gestation period (days)	5.8±1.09[a]	5.5±0.30[b]	5.2±0.11[b]	5.0±0.31[b]	4.8±0.81[b]
Reproductive period (days)	32.3±3.30[a]	35.4±2.12[b]	37.1±3.79[b]	35.6±2.34[a]	35.6±3.11[b]
Number of broods	5.03±0.61[a]	6.8±0.39[b]	7.3±0.59[b]	6.7±0.88[b]	5.2±0.38[a]
Inter-brood duration (days)	4.62±0.48[a]	4.32±0.20[b]	4.26±0.56[b]	4.46±0.39[b]	4.53±0.40[b]
Nauplii/ brood		54.6±2.32[b]	59.38±2.09[b]	58.5±1.82[b]	58.4±2.38[b]
Nauplii released/day	43.26±2.21[a]	11.36±1.29[b]	12.6±2.06[b]	12.41±1.33[a]	12.40±1.45[b]
Cysts/brood	10.29±1.24[a]				
Diameter of cyst (µm)		1.0±0.1[b]	0.8±0.1[b]	0.9±0.1[b]	0.9±0.2[b]
Hatching %	1.2±0.19[a]	239.4±19.4[b]	227.3±18.2[b]	231.4±17.2[b]	242.2±16.1[b]
Fecundity %	241.4±29.4[a]				
Post-reproductive period (days)	54.2±2.07[a]	64.1±2.37[a]	68.2±2.56[b]	68.9±2.46[b]	68.8±2.33[b]
	181.04±7.68[a]	212.8±8.84[b]	251.4±9.43[b]	250.8±7.98[b]	249.9±8.93[b]
	10.3±1.39[a]	10.8±1.56[b]	11.4±1.19[b]	11.1±1.56[b]	10.8±1.37[b]

Figure after ± represents standard deviation; a $p<0.05$; b $p<0.05$

saturated fatty acids (11.7mg), monounsaturated fatty acids (9.2mg) and polyunsaturated fatty acids (12.20mg). Proximate analysis shows that concentration of all the nutritional components increased with increasing dosages of *Sargassum* powder from 250mg to 1000mg (Table-1). Highest concentration of these nutrition components was found at 1000mg, followed by 750mg, 500mg, 250mg and control in a descending order. Dietary components in all the Sargassum treatments were higher than those in the control (rice bran).

Growth Characteristics

The survival percentage of *A. pathenogenetica* fed with *Sargassum* powder was relatively higher in every week compared to the survival percentage at the corresponding time point in the control (Figure 1). At the end of 5th week, the survival percentage in the control was 51.6±2.46% while in Sargassum fed cultures the survival percentage was within the range of 51.9±1.82 - 59.0±1.51%. The highest survival percentage was observed at 500mg followed by 750mg and 1000mg of *Sargassum* powder per day. In the tested experimental duration the variation in survival was statistically significant ($p<0.05$) compared to the control group. The duration taken for 50% mortality of *A. pathenogenetica* fed with rice bran (control) and *Sargassum* powder is shown in Figure-2. In the control feed, *Artemia* took 36.4 days for its reduction into 50% while in *Sargassum* fed cultures this duration was extended up to 42.5 days. The life promoting activity of *Sargassum* increased with increasing dosages from 250mg to 1000mg which gave the maximum result. The statistical significance of variance was ($p<0.05$) compared to the control group. The variation in the life span of *A. pathenogenetica* fed with rice bran (control) and *Sargassum* powder is shown in Figure-3. The maximum life span of *Artemia* in control feed was 90.6±2.09 days while in *Sargassum* fed cultures this duration was extended up to 94.3±2.93 days. The maximum life span increased with increase in the daily dose of *Sargassum* from 250mg to 1000mg; the highest life span was noted at 750mg/day. The statistical significance of variance was ($p<0.05$) compared to the control group. The growth of *Artemia* on 35th day is significantly ($p<0.05$) influenced by different doses of *Sargassum* powder compared to the control feed (Figure-4). The length of *Artemia* in control feed was 83.2±1.61mm while in *Sargassum* fed cultures its length was within the range of 83.4±2.23 - 85.3±2.41mm. The length increased at the daily dose of *Sargassum* from 250mg to 1000mg; the length was maximum at 750mg and 1000mg/day. Figure-5 illustrates the biomass (mg) changes of *A. pathenogenetica* fed with rice bran (control) and different doses of *Sargassum* powder/ day. The biomass of *Artemia* in control feed was 76.3±1.62 mg and at the same time in *Sargassum* fed cultures biomass production increased from 78.4±1.69 to 84.9±1.17mg. The biomass production increased with increase in the daily dose of *Sargassum* from 250mg to 1000mg; the highest life span was noted at 750mg and 1000mg/day. The statistical significance of variance was ($p<0.05$). Figure-6 highlights the variation in the feed conversion rate (FCR) of *A. pathenogenetica* fed with rice bran (control) and *Sargassum* powder. The FCR of *Artemia* in control feed was 69.2±1.68% while in *Sargassum* fed cultures the FCR increased considerably from 70.6±1.64% to 71.4±1.74%. The FCR attained the peak at 750mg dose of *Sargassum* and there was a steady state further. There was no statistical significance of variance ($p>0.05$).

Reproductive Characteristics

Table-2 shows the changes in the reproductive characteristics of *A. parthenogenetica* in response to various dosages of *Sargassum* powder. Maturation period varies considerably with change in habitat conditions, feed types and supplements added to the cultures. The maturation period in the control was 23±2.58 days and it was higher than that in the *Sargassum* treatments. The maturation period decreased from 17.8±0.83 to 15±2.58 days in response to increasing dosage of *Sargassum* powder from 250mg to 1000mg/day. All these dosages had facilitated the early maturation in *A. parthenogenetica* and the maximum influence was shown at 1000mg , followed by 750mg, 500mg and 250mg of Sargassum powder. Dietary supplementation of this seaweed significantly reduced the maturation period in *A. parthenogenetica* ($p>0.05$) compared to the control. Gestation period was significantly ($p<0.05$) influenced by different doses of *Sargassum* powder. The gestation period in the control was 5.8±1.09 days, which slightly decreased from 5.5±0.30 to 4.8±0.81 days in response to increasing dosage of *Sargassum* powder from 250mg to 1000mg/day. Sargassum powder shortened the gestation period in *A. parthenogenetica* and the maximum influence was shown at 1000mg, followed by 750mg, 500mg and 250mg of *Sargassum* powder. The significant variance was ($p>0.05$). The reproductive period in the control was 32.3±3.30 days, and it increases up to 37.1±3.79 (at 500mg Sargassum) and then slightly decreased at 750 and 1000mg dosages. Sargassum powder extended the reproductive period of *A. parthenogenetica* with the statistical significance of ($P<0.05$). The number of broods in the control was 5.03±0.61 days, and it increased up to 7.3±0.59 (500mg *Sargassum*) and then slightly decreased at 750 and 1000mg dosages. *Sargassum* powder increased the number of broods with the statistical significance of ($P>0.05$).

The inter-brood duration in the control was 4.62±0.48, and it decreased up to 4.26±0.56 (500mg *Sargassum*) and then slightly increased at 750 and 1000mg dosages. *Sargassum* powder decreased the inter-brood duration with the statistical significance of ($P>0.05$).

The number of nauplii released per brood in the control was 43.26±2.21, and it decreased up to 59.38±2.09 (500mg *Sargassum*) and then there was a slight steady state at 750 and 1000mg dosages. The significance of variance was ($P>0.05$). On the other hand, the number of cysts released per brood in the control was 1.2±0.19 and it decreased up to 0.8±0.1 at 500mg *Sargassum* and then there was a slight rise in the cyst count at 750 and 1000mg dosages, but less than that in the control. The significance of variance was ($P<0.05$). The diameter of cyst in the control was 241.4±29.4µm, and it decreased up to 227.3±18.2µm at 500mg *Sargassum* and thereafter it increased to 242.2±16.1µm at 1000mg dosage. The statistical significance of variance was ($P>0.05$). Hatching percentage of cysts at increasing dosages of *Sargassum* powder was significantly ($p<0.05$) from the control. The hatching percentage of cyst in the control was 54.2±2.07, and it increased up to 68.2±2.56 at 500mg *Sargassum* and then there was a steady state at 750 and 1000mg dosages. The maximum rise in hatching percentage was noted at 500mg of *Sargassum* powder. The reproductive capacity of *Artemia* in the control was 181.04±7.68%, which increased remarkably up to 500mg *Sargassum* (251.4±9.43) and then there was a gradual decrease in the fecundity to 249.9±8.93%.

The maximum fecundity was observed at 500mg *Sargassum* powder. The statistical significance of variance was ($p>0.05$). The post reproductive period in the control was 10.3±1.39 days and it increased up to 11.4±1.19 days in the presence of 500mg of *Sargassum* powder and then it declined gradually up to 10.8±1.37 days in the presence of 1000mg *Sargassum* powder. The variation in the post reproductive period of *A. parthenogenetica* was significantly different ($p>0.05$) from the control.

DISCUSSION

Nutritional analysis states that rice bran which is considered to be the best feed for *Artemia* contains crude proteins, crude lipids, dietary fibres, all essential amino acids, fatty acids, minerals and some vitamins. 1 gram of Sargassum powder contains crude proteins (12.3%), crude fats (3.4%), dietary fibres (32.2%), essential amino acids such as cystine (2.5mg), histidine (4.5mg), leucine (5.5mg), isoleucine (5.6mg), methionine (5.2mg), phenylalanine (3.7mg), tryptophan (6.4mg), tyrosine (5.3mg), and valaine (5.0 mg), minerals like calcium (67.2mg), phosphate (45.6mg), chloride (23.2mg), sodium (45.1mg), manganese (5.38mg), zinc (1.81mg), potassium (61.3mg), saturated fatty acids (11.7mg), monounsaturated fatty acids (9.2mg) and polyunsaturated fatty acids (12.20mg).

Therefore, dietary supplementation of Sargassum powder provide some amount of these constituents to the basic feed (rice bran), so that nutrient level increased while increasing the dosage of Sargassum powder from 250mg to 1000mg/day. Powder of *S.wightii* contains fatty acids like caproic acid, caprylic acid, capric acid, lauric acid, tridecylic acid, myristic acid, pentadecyclic acid, palmitoleic acid, margaric acid, stearic acid, oleic acid, linoleic acid, γ-linolenic acid, α-linolenic acid, arachidic acid, dihomo-γ-linolenic acid, arachidonic acid, heneicosylic acid, eicosapentaenoic acid, behenic acid, docosahexaenoic acid and lignoceric acid (Vasanthi *et al*., 2003; Visakh Prabhakar *et al*., 2011), of which unsaturated fatty acids (UFAs) like myristic acid, palmitoleic acid, oleic acid, γ-linoleic acid, α-linolenic acid, arachidonic acid and eicosapentaenoic acid are found in larger proportions. Since these UFAs are essential fatty acids for animals, they increase the growth and development of many fishes and animals (Nordoy, 1989; Estevez *et al*. 1999; Evans *et al*. 2000) and at the same time decrease the levels of the expression of cytokines necessary for shifting the function of lymphocytes from the cells mediated response to antibody-mediated response. It is therefore concluded that *Sargassum* powder could improve the dietary values of the basic diet and its effect was increasing with increase in the dosage from 50 mg to 200 mg / day.

Growth is actually the outcome of cell division, enlargement of the new cells and differentiation of body tissues for performing specialized functions in due course of life. Vanhaecke and Sorgeloos (1980) established the fact that the survival and growth rate are mainly determined by the genetic constituents of particular strains growing in different geographical regions of the world, and that, if suitable culture conditions and feeds are given, the genetic control of survival rate can be masked to certain extent in the culture systems. The survival (1%) period of *Artemia* strains in general varied from 66 days (France strain) to 98 days (San Francisco strain) in culture systems without much care (Vanhaecke and Sorgeloos, 1980), which was found to be 90 days for *A. parthenogenetica* used as controls in the present study and about 94.2 days in the *Artemia* fed with the basic diet plus *Sargassum* powder. According to Dana *et al*.(1986), Agh *et al*. (2002), Abatzopoulos *et al*. (2003) and EI-Bermawi *et al*. (2004), survival rate of *Artemia* increased with increase in salinity of water, but it has also been suggested that salinity and temperature together determine the rate of survival and growth in *Artemia* strains (Browne and Hoops, 2000). From the works of Tobias *et al*. (1980) and Lavens *et al*. (1986), it was apparent that nature and type of feed components added to the culture could extend the survival time and rate in *Artemia* strains *in vitro*. Extension of survival time in *Artemia* strains was believed to be due the cumulative effect of nutrients being locked in the feed sources, which may be further prolonged by adding herbal products or ayurvedic drugs that enable *Artemia* to use more of feeds by increasing the feed utilization rate (Mony, 1998). Results of present study confirm that survival and growth rate of *Artemia* increase due to supply of more crude proteins, essential amino acids and fatty acids by the supplementation of *Sargassum* powder, and that while increasing the survival, *Sargassum* powder increases the length and biomass of *Atremia* in the culture. Early works of Tobias *et al*. (1980), Vanhaecke *et al*. (1984), Lavens *et al*. (1986), Balasundaram and Kumaraguru (1987), Gozalbo *et al*. (1987), Vijayaraghavan *et al*. (1987), Bhargava *et al*. (1987) Browne *et al*. (1988), Kitto *et al*. (1991), Marian *et al*. (1991), Basil and Pandian (1991), Indira (1995), Barata *et al*. (1995) and Triantaphyllidis *et al*. (1995) had also confirmed similar findings with different *Artemia* cultures. The attributes of survival and growth are in fact the outcomes of feed conversion rate of the *Artemia* and chemical composition of feed sources supplied to the cultures. Since chemical composition of rice bran is the same in all treatments, the increase in the survival, growth and biomass production were believed to be due to the efficacy of *Sargassum* powder in stimulating feeding rate and feed conversion efficiency in *Artemia* (Indra, 1995; Devi, 1995). Since feed conversion rate declines at 750 and 1000mg of *Sargassum* powder, there was no increase in the growth attributes.

The rate of addition of new individuals to the population of *Artemia* in a culture vessel, is determined by culture condition, feed types and other supplements added to the culture (Immanuel *et al*., 2002). These conditions affect the maturation, gestation period, length of reproductive period, number of broods released by an *Artemia*, resting time between successive broods, number of nauplii released per brood, number of nauplii released per day, number of cysts produced per brood, change in cysts diameter, relative hatching percentage of the cysts, and post reproductive period; even a slight change in one of these attributes produces some marked variations in the other allied attribute/s and hence the reproductive potential itself is changed to a remarkable extent.
Maturation period is the duration of active vegetative phase in which growth occurs logarithmically for biomass production, and it prepares *Artemia* to undergo reproduction that enables it for the production of new individuals for population growth in the culture. Fast growth and early maturation are two main objectives in the *Artemia* culture units to have high rate of biomass production. According to Drewes (2002), the freshly hatched nauplii take 3 weeks to become adults which are about 500 times larger than the nauplii, but the duration of maturation required for the nauplii to pass through metanauplii and post-nauplii stages may vary depending up on the feed

type and culture conditions. Prema and Palavesam (2004) further confirmed that in *A. parthenogenetica* the maturation period was 18 days in control and was reduced to 16days when *Artemia* was fed with the ayurvedic drugs *Whithana somnifera, Cyanodon dactylon* and *Mucuna prurita*. In an experiment, Mony (1998) found that the maturation period was 28.8days in *A. parthenogenetica* and it was reduced to 18 days by *Asparagus racemosus, Mucuna prurita* and *Whitania somnifera* added to the cultures. In the present study, the maturation period of *A. parthenogenetica* was reduced from 23 to 15.2 days. Early maturation denotes fast growth of Artemia as noted by Mony (1998) and Brinda and Palavesam (2002).

Gestation period of *Artemia* is very important in the biology point of view that during this period the young adult *Artemia* stores and exhausts reproductive energy for parturition for the first time while all events of growth are going on continuously. Those drugs or herbal products which promote the growth and reproductive potentialities tend to shorten the gestation period (Leger et al., 1989; Hilda, 1992; John, 1994; Devi, 1995; Indira, 1996; Brintha, 1997; Mageshwari, 1996; Mony, 1998; and Prema and Palaveasm, 2004). In the present study, the gestation period was lesser than that recorded by Mony (1998), Brintha (1997), and Prema and Palavesam (2004). The reproductive period is the reproductive capacity of *Artemia* that determines the number of broods that have to be released by an *Artemia* in its life time (Sorgeloose, 1975; Persoone and Sorgeloose, 1980; Mony, 1998). Lengthy reproductive period results in the release of maximum number of broods, and the length of reproductive period can be extended to some extent by changing the feed composition and by adding nutritional supplements to *Artemia* cultures (Prema and Palavesam, 2004). According to Prema and Palavesam (2004), reproductive period of *A. parthenogenetica* was 36 days in control and was extended to 40 days when *Artemia* was fed with the ayurvedic drugs *Whithana somnifera, Cyanodon dactylon* and *Mucuna prurita* while in yet other experiment, Mony (1998) found that the reproductive period was 41.40 days and it was increased to 47.20 days by *Whitania somnifera* added to the cultures. The present result is in the same line of investigation made by Prema and palavesam (2004).

The number of broods and number of nauplii per brood represent the fertility state of *Artemia* in the culture (Sorgeloos, 1987; Leavens *et al*., 1987. According to Sorgeloos (1987 and 1989), the number of broods per individual increased with increase in the fertility of *Artemia*, which was influenced by feed components (Tobias *et al*., 1980; Gozalbo *et al*., 1987; Bhargava *et al*., 1997) and ayurvedic drugs (Prema and Palavesam, 2004). The number of broods per individual was lower than that recorded by Mony (1998) but higher than those recorded by Prema and Palavesam (2004). Inter-brood duration is the duration taken by the *Artemia* to regain the energy exhausted for the previous brood and to store metabolic energy to be exhausted in the subsequent brood. Experimental evidences from Sorgeloos (1987)and Leavens *et al*., (1987) suggested that the lengthy inter-brood duration denotes poor growth rate which cannot regain reproductive energy quickly while the short inter-brood duration denotes fast growth rate favouring for rapid gaining of reproductive energy. Mony (1998) and Prema and Palavesam (2004) found that the inter-brood duration were 4.62 days in *A. parthenogenetica* and 4.43 days in *A. franciscana*, which were reduced to 4.07 days by Ayurvedic products.

This finding coincides with the results of Browne (1984), Dana (1986), Sorgeloos (1987), Leavens *et al*. (1987), Browne (2000), EI-Bermawi (2001) and Agh (2002 and 2008). The production of more number of new individuals (nauplii) seems to indicate the gaining of more reproductive energy in *Artemia* (Browne, 1984 and Sorgeloos, 1987). Works of Browne (1984), Browne and Hoops (1990), Browne (2000), EI-Bermawi (2001) and Agh (2002 and 2008) concluded that the number of nauplii released per brood varied depending up on habitat conditions and strain types, which was found to be similar in the present cases too. In the present study, the number of nauplii per brood was lower than that recorded by Agh (2002) but slightly higher than that reported by Mony (1998), Prema and Palavesam (2004) and Agh (2008), which might be due to genotypic differences of these strains. The number of nauplii released per day shows the rate of exhaustion of that energy in a day. Browne (1984), Sorgeloos (1987) and Browne and Hoops (1990) suggested that the number of nauplii released per day increases with increase in the available reproductive energy. Therefore, all growth promoting conditions that assist to store more reproductive energy seem to have stimulating effects on the release of the maximum number of nauplii per day while releasing broods (Sorgeloos, 1987; Leavens *et al*. 1987.

The number of nauplii released per day was lower than that recorded by Agh (2002) but slightly higher than that reported by Mony (1998), Prema and Palavesam (2004) and Agh (2008). *Artemia* produces thick-shelled cysts during unfavourable physico-chemical conditions and feed stresses in the culture systems (Personne and Sorgeloos, 1980). When the growth conditions become unsuitable for continuous existence of *Artemia,* the parents secrete a trehalose shell around the eggs and release them as thick-walled cysts (Drinkwater et al., 1991). Further, it is also suggested that temperature (Sorgeloos, 1985), high salinity (Persoone and Sorgeloos, 1980) and feed stresses (Lenz and Dana, 1987) are responsible for cyst production in *Artemia*. In the present case, cyst production was very low as reported by Mony (1998) because of the suitability of *Sargassum* powder as a good supplement. Since *Sargassum* dosage up to 500mg was suitable for growth and reproduction, cyst diameter was low but it increased at 1000mg dosage which might have created some sort of stress in *Artemia*. Such reduction in diameter of cysts was also noticed from the works of Sorgeloos *et al*. (1978) and Triantaphyllidis *et al*. (1996).

Notable increase in the hatching percentage was also observed when *Artemia* cultures treated with some feed components, herbal products and ayurvedic drugs by Vanhaecke *et al*. (1984), Yashiro (1987), Mony (1998), Prema and Palavesam (2004) and Agh (2008). The hatching percentage was lower than that recorded by Agh (2002) but slightly higher than that reported by Mony (1998) and Agh (2008), which might be due to genotypic differences of these strains. Fecundity is very important in the population growth point of view because it determines the rate at which new individuals come to bearing stage for the production of more and more young ones in the culture. In the present study, the fecundity was lower than that recorded by Agh (2002) but slightly higher than that reported by Mony (1998), Prema and Palavesam (2004) and Agh (2008) due to genotypic differences between these strains. Post-reproductive period is the last phase of life cycle which is lasting for a few days before death. The drugs which speeded up the reproductive processes enabled the *Artemia* to exhaust a

large amount of food energy and thereby reducing the post reproductive period of the individual (Mony, 1998). The post reproductive period could be increased by feed components and supplements (Leger *et al*., 1989; Mony, 1998; Prema and Palavesam, 2004). In the present investigation, these post-reproductive periods were lesser than those recorded by Mony (1998) but higher than those recorded by Prema and Palavesam (2002).

Conclusion

These results clearly show that *Sargassum* powder has enough dietary supplementary property to enable Artemia to get faster growth to produce large amount of biomass compared to the normal rice bran feed, and that the growth promoting ability of this powder is increasing with the *Sargassum* dosages up to 500mg/day while higher doses do not have such properties. The supplementing property of *Sargassum* powder is due to the additional crude proteins, dietary fibres, essential amino acids and fatty acids necessary for the growth of *Artemia*. The supplementing effect increases with increasing dosages of *Sargassum* powder, but at higher doses mineral present in it might have created some stress that disturbs the growth and reproductive activities of *Artemia*. Further, this study recommends the growers to use *Sargassum* powder at the rate of 500mg /day along with rice bran feed for the production of more amount of *Artemia* biomass in the production units.

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