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Effect of Ayurvedic Products on the Growth, Survival and Reproduction of *Artemia parthenogenetica* (Abreu-Grobois and Beardmore)

P. PREMA and A. PALAVESAM[1]

P.G. Department of Microbiology
V.H.N.S.N.College
Virudhunagar-626 001
India

[1]*Institute for Coastal Area Studies*
Manonmaniam Sundaranar University
Rajakkamangalam, Nagercoil – 629 502
India

Abstract

The influence of ayurvedic products on the growth, survival and reproduction of *Artemia parthenogenetica* was investigated. Ayurvedic products such as *Withania somnifera* (a), *Mucuna prurita* (b) and *Cinnamomum zeylanicum* (c) in the ratios 1a:1b:1c, 1a:2b:1c, 1a:1b:2c and 2a:1b:1c respectively were used as supplemental feed along with a control feed made out of rice bran and bermuda powder (grass powder; Arugampul; *Cyanodon dactylon*). At each combination the ayurvedic products were given at five different concentrations such as 1.0, 2.0, 3.0, 4.0 and 5.0 $g \cdot l^{-1}$. Optimum concentrations and combinations are suggested.

Introduction

The brine shrimp, *Artemia,* is one of the most widely used live feed organisms in the aquaculture industry and serves as an obligatory feed to ensure maximum survival and growth of early larval shrimp as well as for other marine animals (Sorgeloos 1980; Rao 1994). Ayurvedic products are found to enhance the growth and reproduction in *Artemia*. *Reprostim*, an indigenously prepared ayurvedic product, has been used to induce reproduction in *Artemia franciscana* (Hilda 1992). Several products like *Topal, Selco* and *Lancy* have been used to induce quick fertility in marine crustaceans (Leger et al. 1987). In the present study it is proposed to assess the influence of some new ayurvedic products on the survival, growth and reproduction of *Artemia parthenogenetica*.

Materials and Methods

Adult *A. parthenogenetica* cysts were collected from Thamaraikulam salt pan (08° 04'N to 77° 68'E), India, and were reared in 25 l capacity plastic troughs containing 80 ppt culture medium in the laboratory. Rice bran suspension was given as feed twice daily to the culture stock. Freshly hatched nauplii from this stock culture were used for the experiment. To find out the effect of ayurvedic products on the growth performance and reproduction in *A. parthenogenetica*, the experiment was carried out simultaneously in triplicates with control. During the experimental period 25 freshly hatched *Artemia* nauplii from the initial stock were cultured in glass bowls with 200 ml of high saline water (80 ppt). Rice bran and bermuda powder (1:1; 200 $g \cdot l^{-1}$) were used as control feed. Finely powdered ayurvedic products (20 mm) like *Withania somnifera* (a), *Mucuna prurita* (b) and *Cinnamomum zeylanicum* (c) were used in the combination of 1a:1b:1c (C_1), 1a:2b:1c (C_2), 1a:1b:2c (C_3) and 2a:1b:1c (C_4) as supplemental feed with control. In each combination, a stock solution (200 $g \cdot l^{-1}$) was prepared. From this stock solution 1, 2, 3, 4 and 5 ml were used for feeding with respect to five different concentrations (1.0, 2.0, 3.0, 4.0 and 5.0 $g \cdot l^{-1}$) **(Table 1)?**. During feeding, the respective concentrations of the nutrient suspension were completely squeezed through a 60 µm mesh and fed freshly at *ad libitum*. Salinity was monitored daily in the morning and was maintained at the optimum level (80 ppt). Every day dead individuals and the faeces were removed prior to feeding. Water quality in each experiment was maintained at the optimum level by exchanging the water daily as well as by providing mild aeration through an air pump.

Growth, survival and the reproductive characteristics of the experimental animals were recorded. Survival was calculated by recording the daily mortality. As an index of growth, the length of *Artemia* was measured at random sampling in alternate days with least disturbance and were returned immediately to the experimental culture. Then by using the regression equation, Log weight = -1.6021 + 2.4135 x Log length, the corresponding weight was measured. The reproductive rate was determined by counting the number of nauplii released by individual *Artemia* at the tested experimental diets. For this, five individuals of mature *Artemia* in each combination of all

Table 1. Composition and combination of supplemental diets.

Combination	Ayurvedic products (ratio)			Concentrations ($g.l^{-1}$)				
	W.S	M.P	C.Z	1.0	2.0	3.0	4.0	5.0
1	1a	1b	1c	,,	,,	,,	,,	,,
2	1a	2b	1c	,,	,,	,,	,,	,,
3	1a	1b	2c	,,	,,	,,	,,	,,
4	2a	1b	1c	,,	,,	,,	,,	,,
Control feed	-	-	-	-	-	-	-	-

W.S= *Withania somnifera* (a); M.P =*Mucuna prurita* (b); C.Z=*Cinnamomum zeylanicum* (c)

the nutrient concentrations were transferred to separate bowls and the number of cysts and nauplii released were recorded. From this value, average number of cysts and nauplii released at the tested nutrient concentrations of all the combinations were calculated. The results obtained in the experiment were subjected to appropriate statistical analysis following Zar (1984).

Results

Growth rate

The growth of *A. parthenogenetica* reared in different nutrient sources is given in figure 1. Maximum growth of 9.10 ± 0.49 mm, 7.50 ± 0.20 mm, 9.72 ± 0.74 mm and 9.40 ± 0.48 mm were recorded on the 15th day at 3.0 g·l^{-1} concentration of 1st , 2nd, 3rd and 4th combinations, respectively. Among the four tested combinations, the 1a:1b:2c ratio at 3.0 g·l^{-1} concentration, was found to be optimal for maximizing the growth. The correlation co-efficients observed for the length and weight of *A. parthenogenetica* fed with the tested combinations were significantly different (r = 0.939– 0.968; P < 0.05; Table 2).

Survival rate

The survival of *A . parthenogenetica* under different nutrient sources is given in figure 2. Combinations, C_1, C_2, C_3 and C_4 gave good survival rates of 85, 72, 92 and 83% respectively on the 15th day at 3.0 g·l^{-1} concentrations. The maximum survival rate was exhibited mostly at 3.0 g·l^{-1} concentrations. Among the four tested combinations, the ayurvedic products at the ratio of 1a:1b:2c was found to be optimum for maximizing the survival rate.

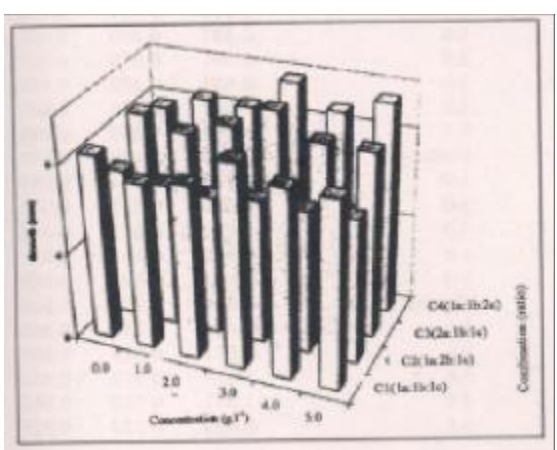

Fig. 1. Growth of *A. parthenogenetica* fed with different combinations of ayurvedic at various concentrations

Reproduction

The ovarian development of *A. parthenogenetica* reared in four different combinations are given in figure 3. In the control group, the ovarian development was visible on the 18th day. But in the experimental groups, the ovarian development was faster in three combinations (C_1,C_3 and C_4) and it was slow at combination 2. Compared to control diet fed group, the ovarian development in experimental group of all concentrations in combination 4 was significantly different ('t' value 2.89 to 6.36; $P<0.05$); whereas, in other combinations (C_1, C_2 and C_3) it was not significant ($P>0.05$). *A. parthenogenetica* released two broods in each combinations 1, 3 and 4 and five broods in combination 1. Among the four tested combination, the maximum number of nauplii released in the first brood was 59.00 ± 12.26 in 1a:1b:1c ratio at 3.0 $g \cdot l^{-1}$ concentration. Minimum number of nauplii

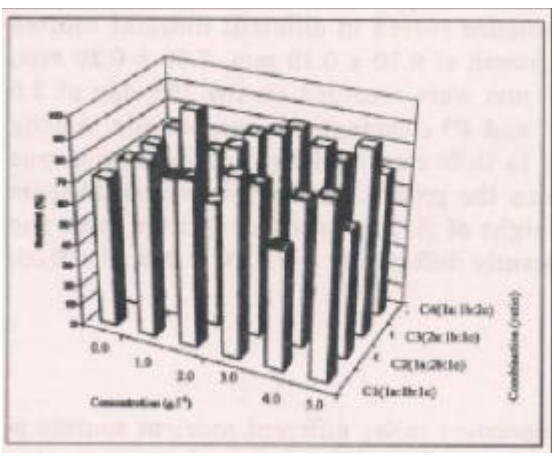

Fig. 2. Survival of *A. parthenogenetica* fed with different combinations of ayurvedic at various concentrations

Table 2. Summary of correlation co-efficient made on *A. parthenogenetica* fed with different combinations of ayurvedic products.

Combinations	Parameters compared	Concentrations ($g \cdot l^{-1}$)	Regression value		
			a	b	r
C_1(1a:1b:1c)	Length and Weight	Control	-0.090	0.387	0.952
		2.0	-0.845	0.485	0.951
		3.0	-1.175	0.587	0.939
		4.0	-0.992	0.532	0.939
		5.0	-0.953	0.518	0.935
C_2(1a:2b:1c)	Length and Weight	Control	-0.682	0.417	0.954
		1.0	-0.597	0.399	0.967
		2.0	-0.561	0.379	0.968
		3.0	-0.624	0.402	0.966
		4.0	-0.572	0.384	0.963
		5.0	-0.564	0.383	0.962
C_3(1a:1b:2c)	Length and Weight	Control	-0.797	0.488	0.958
		1.0	-0.997	0.544	0.954
		2.0	-0.788	0.513	0.946
		3.0	-0.922	0.571	0.934
		4.0	-0.983	0.583	0.937
		5.0	-0.029	0.592	0.939
C_4(2a:1b:1c)	Length and Weight	Control	-0.019	0.583	0.938
		1.0	-0.875	0.513	0.968
		2.0	-0.974	0.526	0.963
		3.0	-1.058	0.572	0.956
		4.0	-1.041	0.562	0.952
		5.0	-0.886	0.522	0.957

released in the first brood was 23.60 ± 5.28 in 1a:1b:2c ratio at 3.0 g·l^{-1} concentration (Table 3). Maximum number of nauplii released in the second brood was 84.00 ± 0.00 in 1a:2b:1c ratio at 2.0 g·l^{-1} concentration against 20.00 ± 4.08 at 1a:1b:1c ratio. Maximum number of cysts released in the second brood was 46.00 ± 11.34 in 1a:1b:2c ratio at 2.0 g·l^{-1} concentration.

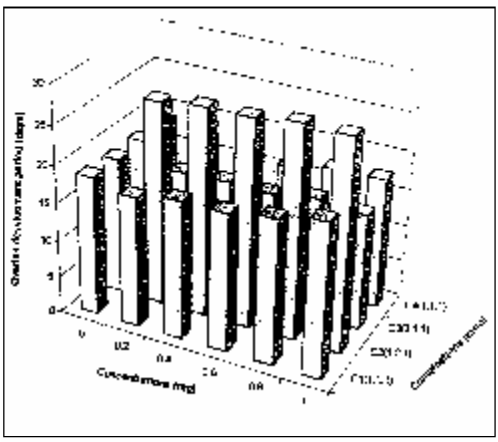

Fig. 3. Time taken for the development of ovary by *A. parthenogenetica* fed with different combinations of ayurvedic, products in various concentrations

The third brood of *A. parthenogenetica* released both nauplii and cysts. The maximum number of nauplii released at 5.0 g·l^{-1} concentration was 65.20 ± 39.96 against 26.70 ± 5.25 at 2.0 g·l^{-1} concentration. The maximum

Table 3. Number of offspring released in each brood of *A.parthenogenetica* fed with different concentrations of ayurvedic products *W.somnifera* (a), *M.prurita* (b), *C.zeylanicum* (c) at different combinations. Each value is the mean of five individual observations.

Events	Combinations of ayurvedic products	Off springs	Mean value (X)					
			Control	Concentrations of ayurvedic products (g·l^{-1})				
			0.00	1.0	2.0	3.0	4.0	5.0
Number of offspring in the first brood	C_1 (1a:1b:1c)	Nauplii	47.8	34.2	42.0	59.0	47.0	41.0
	C_2 (1a:1b:1c)	Nauplii	38.4	45.8	46.6	37.2	33.2	37.2
	C_3 (1a:1b:1c)	Nauplii	30.4	37.2	30.0	23.6	39.8	28.2
	C_4 (1a:1b:1c)	Nauplii	30.5	42.4	40.6	28.8	48.4	44.0
Number of offspring in the second brood	C_1 (1a:1b:1c)	Nauplii	20.0	27.3	27.0	41.2	32.8	33.5
	C_2 (1a:1b:1c)	Cyst	18.5	28.0	16.0	00.0	00.0	49.0
	C_3 (1a:1b:1c)	Nauplii	51.7	77.0	84.0	25.7	41.8	58.4
	C_4 (1a:1b:1c)	Cyst	27.5	30.0	34.0	28.0	22.0	00.0
		Nauplii	29.2	54.0	57.0	71.0	59.3	61.2
		Cyst	00.0	24.0	46.0	19.5	34.0	00.0
		Nauplii	29.2	44.0	39.0	64.0	30.5	71.0
		Cyst	00.0	54.6	32.6	15.3	24.0	15.0
Number of offspring in the third brood	C_1 (1a:1b:1c)	-	00.0	00.0	00.0	00.0	00.0	00.0
	C_2 (1a:1b:1c)	Nauplii	49.0	54.8	26.7	31.6	30.7	65.2
		Cyst	26.0	10.0	17.5	00.0	14.0	00.0
	C_3 (1a:1b:1c)	-	00.0	00.0	00.0	00.0	00.0	00.0
	C_4 (1a:1b:1c)	-	00.0	00.0	00.0	00.0	00.0	00.0
Number of offspring in the fourth brood	C_1 (1a:1b:1c)	-	00.0	00.0	00.0	00.0	00.0	00.0
	C_2 (1a:1b:1c)	Nauplii	40.6	48.2	26.4	35.2	37.8	58.8
	C_3 (1a:1b:1c)	-	00.0	00.0	00.0	00.0	00.0	00.0
	C_4 (1a:1b:1c)	-	00.0	00.0	00.0	00.0	00.0	
Number of offspring in the fifth brood	C_1 (1a:1b:1c)	-	00.0	00.0	00.0	00.0	00.0	00.0
	C_2 (1a:1b:1c)	Nauplii	30.3	27.8	54.8	42.2	28.6	29.2
	C_3 (1a:1b:1c)	-	00.0	00.0	00.0	00.0	00.0	00.0
	C_4 (1a:1b:1c)	-	00.0	00.0	00.0	00.0	00.0	00.0

number of cysts released in the control group was 26.00 ± 0.00 and the minimum number of cysts released at 1.0 $g \cdot l^{-1}$ concentration was 10.00 ± 0.00 (Table 3). The fourth and fifth brood released only nauplii. The maximum number of nauplii released in the fourth and fifth broods at 5.0 and 2.0 $g \cdot l^{-1}$ concentrations were 58.80 ± 33.12 and 54.80 ± 15.38 respectively. The minimum number of nauplii released at 2.0 $g.l^{-1}$ and 1.0 $g \cdot l^{-1}$ concentrations were 26.40 ± 6.92 and 27.80 ± 15.38, respectively (Table 3).

Compared to the control group, the variations in nauplii released between fourth and fifth broods were not significantly different (Student 't' test; $P>0.05$). But the variation in cysts released between the third and second brood was significantly different (Student 't' test; $P<0.05$) at 4.0 $g \cdot l^{-1}$ concentration alone and not significant in the other four different concentrations (1.0, 2.0, 3.0 and 5.0 $g \cdot l^{-1}$) as well as in between all concentrations in first, fourth and fifth brooders. Corresponding to the variations in ovarian development, release of nauplii and cysts, the time taken between two successive spawns also varied much between those fed with control and experimental diets (Table 4).

Total life span

Total life span of *A. parthenogenetica* varied with different concentrations of ayurvedic products and the data are presented in figure 4. For those animals fed with control diet the average life span was 27.80 ± 0.75 days. The maximum life span exhibited was 48.20 ± 0.98 days in 1a:2b:1c ratio at 5.0 $g \cdot l^{-1}$ concentration. The minimum life span exhibited was 27.20 ± 2.14 days in 1a:1b:1c ratio at 2.0 $g \cdot l^{-1}$ concentration. The differences in total life span was significantly different ('t' test; $P< 0.05$) only at 2.0 $g \cdot l^{-1}$ concentration under 1a:1b:2c ratio. Whereas, it was not significant ('t' test; $P> 0.05$) at three different combinations (C_1, C_2 and C_4).

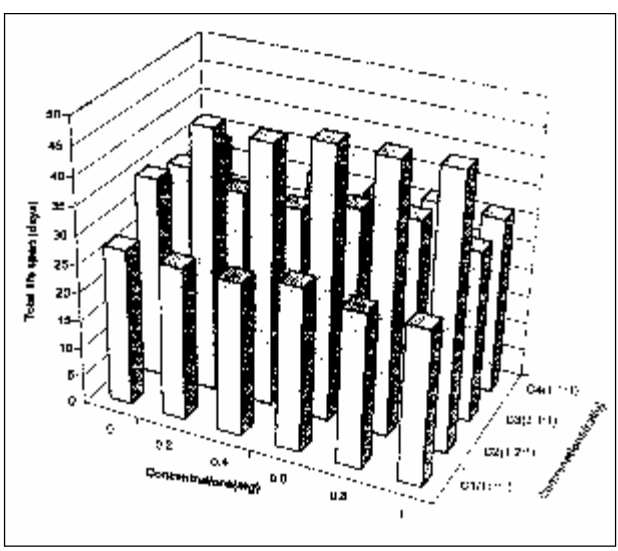

Fig. 4. Total life span of *A. parthenogenetica* fed with different combinations of ayurvedic, products as a function of concentrations

Discussion

The present study offers promising possibilities of using ayurvedic products as suitable feed additives in *Artemia* culture. Dobbeleir et al. (1980) have also reported the use of nonsoluble waste products from agricultural crops or from food processing industries such as rice bran, corn bran, soybean pellet, lactoserum, sugarcane, molasses as feed for high density culture of *Artemia*. It is obvious that, supplemented diets with ayurvedic products influenced the survival of *A. parthenogenetica*. One interesting thing is that the *Artemia exh*ibited maximum survival at 3.0 $g \cdot l^{-1}$ concentration and minimum at 4.0 $g \cdot l^{-1}$ concentration in all tested combinations. The possible reason for low survival in control and other concentrations as indicated by Hilda (1992) may be due to overfeeding of food particles on the thoracopods.

From the present observations, it is evident that, bermuda powder, rice bran supplemented with ayurvedic products at combination 4 was found to be optimal for better survival and growth of *A. parthenogenetica*. Ayurvedic products have generally been found more effective in supporting larval growth and survival (Devi 1995). However, as this product is being used

Table 4. Time interval (days) between two successive broods of *A. parthenogenetica* fed with different concentrations of ayurvedic products *W. somnifera* (a), *M. prurita* (b), *C. zeylanicum* (c) at different combinations. Each value is the mean of five individual observations.

Events	Combinations of ayurvedic products	Mean value (X)					
		Control	Concentrations of ayurvedic products ($g \cdot l^{-1}$)				
		0.0	1.0	2.0	3.0	4.0	5.0
Time interval between ovary development and first brood (days)	C_1 (1a:1b:1c)	3.8	3.4	4.4	4.2	5.2	4.8
	C_2 (1a:1b:1c)	3.2	3.4	3.4	3.2	3.0	3.0
	C_3 (1a:1b:1c)	3.6	4.4	3.6	4.0	3.8	4.2
	C_4 (1a:1b:1c)	3.6	3.6	3.8	3.6	3.4	3.4
Time interval between first and second brood (days)	C_1 (1a:1b:1c)	6.8	6.8	7.0	7.2	7.0	7.2
	C_2 (1a:1b:1c)	4.8	4.8	6.2	6.4	5.4	5.8
	C_3 (1a:1b:1c)	5.2	5.2	4.2	5.2	4.4	4.2
	C_4 (1a:1b:1c)	6.8	6.8	6.8	5.4	6.2	6.4
Time interval between second and third brood (days)	C_1 (1a:1b:1c)	0.0	0.0	0.0	0.0	0.0	0.0
	C_2 (1a:1b:1c)	3.2	3.2	3.6	3.0	3.2	3.4
	C_3 (1a:1b:1c)	0.0	0.0	0.0	0.0	0.0	0.0
	C_4 (1a:1b:1c)	0.0	0.0	0.0	0.0	0.0	0.0
Time interval between third and fourth brood (days)	C_1 (1a:1b:1c)	0.0	0.0	0.0	0.0	0.0	0.0
	C_2 (1a:1b:1c)	2.4	2.6	2.8	2.6	2.8	2.4
	C_3 (1a:1b:1c)	0.0	0.0	0.0	0.0	0.0	0.0
	C_4 (1a:1b:1c)	0.0	0.0	0.0	0.0	0.0	0.0
Time interval between fourth and fifth brood (days)	C_1 (1a:1b:1c)	0.0	0.0	0.0	0.0	0.0	0.0
	C_2 (1a:1b:1c)	2.4	3.0	3.4	2.4	2.8	3.4
	C_3 (1a:1b:1c)	0.0	0.0	0.0	0.0	0.0	0.0
	C_4 (1a:1b:1c)	0.0	0.0	0.0	0.0	0.0	0.0

with the aim to stimulate the reproductive potential, the optimum concentration has to be finalized after knowing the influence of the product on reproduction.

Ayurvedic products improving the reproductive potential in animal have been established. *Withania somnifera* (a), *Mucuna purita* (b), *Cinnamomum zeylanicum* (c), *Asparagus racemosus, Abroma angusta, Accacia arabica, Alpinia galanga, Areca catechu, Butea monosperma, Aristolochia bracteata, Carica papaya, Gloriosa superba, Ocimum barilacum* etc. have been used to induce reproduction in vertebrates (Dastur 1962; Joshi and Magar 1952 and Chopra et al. 1956). Ayurvedic products have also been tested in lower invertebrates by several authors (Hilda 1992; Devi 1995). Hilda (1992) fed the reprostim to *A. franciscana* and found that the effect of the product and the reproduction rate was highly pronounced during the early stages of the brine shrimp and in the later period the effect was found to be low. In a study on the effect of *Asparagus sp.* in combination with rice bran Devi (1995) reported that the enhanced reproduction and related parameters in *A. franciscana.* In the present study feeding *W. somnifera* (a), *M. prurita* (b), *C. zeylanicum* (c) in different combinations (C_1-C_4) to the brine shrimp *A. parthenogenetica* in a wide range of concentrations starting from 1.0 $g \cdot l^{-1}$ to 5.0 $g \cdot l^{-1}$ along with rice bran and bermuda powder revealed that the optimum concentration of 3.0 $g \cdot l^{-1}$ maximized the reproduction as well as other related functions. Interestingly an initial inhibition of reproduction in combination 2 was also noticed in the present study. This might have helped the brine shrimp to prolong its life period up to 47 days. Whereas, the other combinations seem to reduce the maturation time from 18 to 16 days and succumbed within a month. These short periods of survival could produce only two broods; whereas, combination 2 could release their young ones in 5 broods. In all cases, in the initial brood, only nauplii were released and subsequently in the second brood, the nauplii releasing mechanism was shifted to the cyst releasing mechanism perhaps due to a kind of stress by the ayurvedic products. If the stress is due to the ayurvedic products, the dominating effect of *M. prurita* (b) in the ratio of 1a:2b:1c combination might have again nullified the stress inducing factor and prolonged the process of releasing nauplii in the late successive broods.

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