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Culture of rotifer *Brachionus angularis* Hauer feeding with dried *Chlorella*

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Abstract : A study was carried out to know the performance of powdered dried Chlorella for culture of rotifer Brachionus angularis. B. angularis was fed with powdered dried Chlorella in treatment 1, live or fresh cultured Chlorella in treatment 2 and baker's yeast in treatment 3. The rage of physicochemical parameters during culture of rotifer such as water temperature, air temperature, pH and dissolved oxygen were within the suitable ranges for B. angularis culture and more or less similar in all the treatments. The highest population densities of B. angularis recorded in treatment 1, treatment 2 and treatment 3 were 50, 60 and 30 ind/ml, respectively. The mean population densities were 30.1±12.2, 37.4±14.6 and 21.1±6.1 ind./ml in treatment 1, treatment 2 and treatment 3, respectively. The results revealed that the powdered dried Chlorella had significant effect on the population density of B. angularis and was better than that of baker's yeast. So, when live Chlorella will not be available, powdered dried Chlorella can be successfully used as feed for B. angularis culture.

Keywords: Rotifer, Chlorella, yeast, culture medium

Introduction

Zooplankton plays an vital role in the food chain of fish as animal food, which supply amino acids, fatty acids, vitamins, minerals, etc. (Watanabe *et al.,* 1983). In spite of different efforts to replace live food by inert feeds, the rearing of fry and juvenile of fishes (specially zooplankton feeder) in nursery ponds mostly depend on the greater abundance of zooplankton (Habib *et al.,* 1988). Offering the correct food at optimal densities for each larval stage is necessary for efficient and economical hatchery operation. In general, rotifer has both nutrient content and a high rate of daily production (Lubzens, 1987). Rotifer transmits adequate supplies of micro and macronutrients, vitamins and even antibodies to fish larvae (Gastesoupe, 1982). The level of polyunsaturated ω-3 fatty acid in rotifer is believed to affect both survival and growth rate of fish larvae (Koven *et al.,* 1990). A brackish water rotifer *B. plicatilis* has been used as food for marine fish larvae and planktonic crustaceans through out the world (Watanabe *et al.,* 1983). The culture of freshwater fish having a small planktonic larval stage requires a suitable food source, such as living rotifers. In contrast to the brackish water rotifer, freshwater rotifer has gained limited practical significance and their use as a starter food for rearing fish is still less developed. In freshwater aquaculture, *B. calyciflorus* and *B. rubens* have been used as food for fish larvae and their limited use is probably due to convenient inert food being available to feed freshwater fish larvae (Groeneweg & Schluter, 1981). In order to attain stable mass production of rotifer, it is desirable to develop a food source that will support rotifer growth completely by itself. Since large scales algal production is relatively cheap, various types of algae are routinely being produced for feeding rotifers. However, sometimes, algal cultures crash which may in turn lead to problems of finding adequate food for rotifers. Therefore, stored algae such as dried algae could be used as a substitute. On the other hand, at times there may be excessive production of microalga, which could be stored for future use (Martinez & Chavez, 1994). The nutritional quality as well as the digestibility of the stored-microalga may vary considerably and consequently the growth responses of zooplankton feeding on them (Dobberfuhl & Elser, 1999). In this context, population level responses of rotifer to stored microalgae have not been well documented. *B. angularis* is an important freshwater rotifer. Thus, considering the above facts, the present study was undertaken to culture rotifer *B. angularis* using powdered dried *Chlorella*.

Materials and methods

Preparation of culture medium for Chlorella: Inexpensive organic culture medium was prepared with 700 g of pulse bran *(Vigna mungu)* mixed in 20 L tap water in a plastic bucket. Then, after 1 week, 11 g urea was added to each bucket. After 4 weeks partially decomposed pulse bran mixture was filtered through thin mark in cloth and solid materials were discarded. The supernatant was siphoned to another bucket after

another week and 2 g lime (CaO) per liter of medium was mixed to make it clear and pH was adjusted to 7 adding H_2SO_4. Then, after a week the clear supernatant was again siphoned to another bucket and this clear solution was ready as the algal culture medium. The prepared medium was sterilized in an autoclave (at 121^0C) for 15-20 minutes.

Chlorella seed collection and culture: For the collection of algal seed, pond water was collected and centrifuged (2000 rpm for 3 min.) to collect dense sample of algae. This dense sample of algae was examined under a compound microscope to check whether there were any probable culturable microalgae. Microalgae were first collected in a 5-10 ml medium in test tube. Then about 5 days prospective cultures were examined and used as seed if *Chlorella* were found in dominant condition. Seed *Chlorella* was cultured in 500 ml conical flask with 200 ml sterilized medium. The culture was done in sufficient natural light and temperature.

Preparation of powdered Chlorella: Cultured *Chlorella* was centrifuged (2000 rpm for 5 minutes) around 13th days of culture. Then the dense sample was preserved in the refrigerator. The frozen *Chlorella,* after thawing, was dried in a microwave oven. Then it was powdered with a mortar and pestle. The powdered *Chlorella* was preserved in a bottle for further use.

Rotifer stock culture: The seed of rotifer was collected from a pond through selective netting with zooplankton net. The seed was cultured for 2 months in plastic jars of 5 L capacity with continuous aeration to maintain stock culture. Fresh cultured *Chlorella* was used as food during the maintenance of stock rotifer culture.

Experimental culture of rotifer: Rotifer *(B. angularis)* were cultured in 3 L size plastic jars containing 1 L water. All the jars were stocked with *B. angularis* (from stock rotifer culture) at the initial density of 10 rotifer/ml. The experiment was conducted with three treatments each treatment with 3 replications. Powder dried *Chlorella* (0.1 g/L water), fresh *Chlorella* ($1.2x10^6$ cells/ml water) and baker's yeast (0.15 g/L water) were used as feed for rotifer in treatment 1, treatment 2 and treatment 3, respectively. All the jars were supplied with continuous aeration for 24 hours to maintain adequate dissolved oxygen level. Continuous lighting for 24 hours was arranged for better growth of *B. angularis*. Water temperature, air temperature, pH, DO, etc. were monitored regularly. Density of *B. angularis* was determined daily with a zooplankton counting cell under a compound microscope.

Statistical analysis: Data were subjected to analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) for significance in variance.

Results and Discussion

Result of cell density of *Chlorella* and environmental factors during culture period are shown in Table 1. The range of cell density of *Chlorella* was $1.3x10^6$ to $4.5x10^6$ cells/ml which was similar to that found by Rahman *et al.* (2005). The environmental factors during the culture period were favorable for *Chlorella* culture and that also were more or less similar to that found by Rahman *et al.* (2005). The average air temperature and water quality parameters during *B. angularis* culture are shown in Table 2. The optimum water temperature for rotifer growth as reported by McVey & Moore (1983) was 22 to 30^0C. In the present study, the lowest and the highest temperature were 20^0C and 24.5^0C, respectively. Hirayama & Kusano (1972) reported that optimum temperature for rotifer culture was around 25^0C. Mean pH values of the water of rotifer cultures under three treatments were about neutral (6.86-7.10). Furukawa & Hidaka (1973) reported that rotifers grow well in stable pH. Hoff & Snell (1989) reported an optimum pH range for *B. calyciflorus* at 6.0-8.0 with upper and lower limits of 9.5 and 4.5, respectively.

Data of population density of *B. angularis* are presented in Table 3. The mean value of *B. angularis* fed on powdered dried *Chlorella* under treatment 1 was 30.11±11.99 ind./ml. Studies on the use of preserved algae for zooplankton growth are rare because of a general idea that non-living algae do not support their growth. However, Baer & Goulden (1998) found the population density for *B. calyciflorus* cultured with heat-killed *Chlorella* ranged from 6±1 to 26±6 ind./ml. This result was more or less similar to that of the present study although the species of rotifer was different.

The mean value of *B. angularis* density fed on fresh cultured *Chlorella* under treatment 2 was 37.44±14.6 ind./ml. This result was close to the findings of Rezeq & James (1987) in case of *B. plicatilis*. Hirayama & Nakamura (1976) reported that live algae supported the best growth of rotifer. The mean value of *B. angularis* fed on baker's yeast under treatment 3 of the present experiment was the lowest, 21.11±6.11 ind./ml. Rahman *et al.* (1993) found that the mean values of B. *calyciflorus* fed on baker's yeast was 24.17±9.40 ind./ml which was more or less similar to that of the present study although the species of rotifer was

different. It is reflected from the present study that the mean population density of *B. angularis* was the highest under treatment 2 that was fed on fresh cultured *Chlorella.* The mean population density of *B. angularis* was higher under treatment 1 that fed on powdered dried *Chlorella* under treatment 1 feed on baker's yeast and was close to treatment 2. This indicated that fresh cultured or live *Chlorella* were the best as food for rotifer *B. angularis* and dried powdered *Chlorella* were also better than baker's yeast as food for the rotifer. Hiayama & Nakamura (1976) found that the dried *Chlorella* in suspension was less effective for growth of *B. plicatilis* than living *Chlorella*, but the dried *Chlorella* was much more effective than a suspension of yeast at the same density. One of the reasons for the slightly lower production of *B. angularis* fed on powdered dried *Chlorella* under treatment 1 than that fed on fresh cultured live *Chlorella* under treatment 2 was the acclimation of rotifer with live *Chlorella* as feed in the stock culture from where the rotifer were taken into the experimental jars. Another reason is that when dried, algal digestibility probably would increase but the nutritional quality may decrease (Brown, 1995).

It may be concluded that, as the powdered dried *Chlorella* is better than baker's yeast as feed and it has significant effect on the population density of *B. angularis*, so, when live *Chlorella* will not be available, powdered dried *Chlorella* can be successfully used as a feed for *B. angularis* during its culture.

Table 1. The cell density of *Chlorella* cultured in inexpensive organic medium along with environmental factors during a period of 18 days.

Culture Duration (days)	Cell Density ($x10^6$ ells/ml)	Light intensity (Lux)	Water temp. (°C)	Air temp. (°C)	Sunshine period (hrs)	Rainfall (mm)
2	1.32	3750	26.5	27.0	8.8	0.0
3	1.65	3650	26.0	27.0	9.5	0.0
4	2.19	3750	25.5	27.0	8.8	0.0
6	2.36	3750	26.0	27.5	8.1	0.0
7	2.49	3250	26.0	27.0	8.2	0.0
8	2.86	3650	25.0	26.5	5.2	0.0
9	3.01	3250	24.5	26.0	8.0	0.0
10	3.05	2970	24.5	26.0	9.3	0.0
11	3.22	3100	24.0	25.5	9.5	0.0
13	4.49	4120	24.5	26.0	8.9	0.0
14	4.11	3000	25.0	26.5	9.2	0.0
15	3.79	3500	25.0	26.5	9.5	0.0
16	3.29	2850	25.0	26.5	9.3	0.0
17	2.87	3150	25.5	26.0	8.0	0.0
18	2.67	2900	24.5	25.5	9.5	0.0
Mean±sd	2.89 ± 0.85	3376 ± 387.9	25.2 ± 0.7	26.4 ± 0.6	8.7 ± 1.1	0.0

Table 2. Mean values (±sd) of air temperature, water temperature, pH and dissolved oxygen under 3 treatments during culture of *B. angularis*

Factors	Treatments*		
	T_1	T_2	T_3
Air temperature (°C)	24.7±0.3	24.7±0.3	24.7±0.3
Water temperature (°C)	22.5±0.9	22.4±0.8	22.5±0.9
pH	6.93±0.03	7.00±0.07	6.97±0.05
Dissolved oxygen (mg/L)	6.16±0.51	6.12±0.61	6.11±0.57

*T_1=Powdered dried *Chlorella*,
T_2=Fresh cultured *Chlorella*,
T_3=Baker's yeast.

Table 3. Population density (ind./L) of the rotifer *B. angularis*
cultured under three treatments*

Duration of culture (days)	Population density (ind./ml)		
	T_1	T_2	T_3
1	16	18	13
2	21	24	15
3	22	25	16
4	23	29	18
5	24	37	20
6	30	41	24
7	40	49	26
8	45	54	28
9	50	60	30
Mean±s.d.	30.1±12.0b	37.4±14.6a	21.1±6.1c
Range	16 to 50	18 to 60	13 to 30

*T_1=Powdered dried *Chlorella*, T_2=Fresh cultured *Chlorella*, T_3=Baker's yeast. Data presented are averages of three replications. Data in the same raw bearing different letter are significantly different ($p<0.05$).

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