ISSN: 2224-0616
Int. J. Agril. Res. Innov. & Tech. 3 (2): 41-48, December, 2013 Available online at http://www.ijarit.webs.com

SUITABILITY OF *Chlorella ellipsoidea* AS FOOD FOR PRODUCTION OF THE ROTIFER *Brachionus calyciflorus*

S. Akter[1], M. Shahjahan[1*], M.S. Rahman[1] and P.S. Das[2]

Received 7 August 2013, Revised 10 December 2013, Accepted 25 December 2013, Published online 31 December 2013

Abstract

Rotifers are valuable live food for larval fish and crustacean in aquaculture. In the present study, we conducted an experiment to evaluate the suitability of *Chlorella ellipsoidea* as food for production of the rotifer, *Brachionus calyciflorus*. The experiment was carried out in three treatments using dried powder *Chlorella ellipsoidea* (T1), fresh live *Chlorella ellipsoidea* (T2) and Baker's yeast (T3) as food with three replications. For supplying food to rotifers, *C. ellipsoidea* was cultured sufficiently in different inexpensive culture media before rotifer culture and it was continued up to the end of the rotifer culture experiment. The ranges of environmental factors analyzed were suitable for both *C. ellipsoidea* and rotifers culture during the experimental period. Maximum cell densities of *C. ellipsoidea* were recorded in inexpensive pulse bran extract medium during the culture period. The mean population densities (means ± SEM) of *B. calyciflorus* were significantly ($p<0.01$) highest in T2 (28.6 ± 4.64 (x10^3) individuals L^{-1}) compared to T1 (11.6 ± 1.24 (x10^3) individuals L^{-1}) and T3 (11.4 ± 1.82 (x10^3) individuals L^{-1}). The findings of present study revealed that fresh cultured *C. ellipsoidea* was the best food for production of the rotifer, *B. calyciflorus*.

Keywords: Rotifer, *Chlorella*, Aquaculture, Culture Medium, Water Quality

[1]Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]FAO-BOBLME-BFRI Hilsa Project, Bangladesh

*Corresponding author's email: mdshahjahan@bau.edu.bd (M. Shahjahan)

Introduction

Rotifers are multicellular animals with body cavities that are partially lined by mesoderm. These organisms are valuable live food for larval fish and crustacean culture. Several characteristics of rotifers, including their nutritional quality, body size and relatively slow motility have contributed to their usefulness as good prey for active larvae (Snell and Carrillo, 1984). In general, rotifers have both nutrient content and a high rate of daily production (Lubzens, 1987). Rotifer transmits adequate supplies of micro and macronutrients, vitamin and even antibodies to the fish larvae (Gatesoupe, 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). Rotifer forms an excellent initial food because of its appropriate size (130-320 µm), planktonic nature, rapid production rate, suitability for mass culture under controlled conditions, ability to grow and reproduce in high density cultures and the possibility of artificially manipulating its nutritional qualities along with the euryhaline nature (Dhert *et al.*, 2001). Among different species of rotifer, *Brachionus* has been widely used as essential food source in raising marine fish, shrimp and crab larvae due to its tolerance to the marine environment (Lubzens, 1987; Cheng *et al.*, 2004). Several researchers in different countries of the world have been conducted researches on rotifer culture, enrichment of rotifer culture, development of rotifer culture methods for the purpose of fish larvae feeding for improvement of fish culture industry (Lubzens *et al.*, 2001; Dhert *et al.*, 2001; Hagiwara *et al.*, 2001; Leschenko *et al.*, 2005; Arimoro, 2007; Ludwig *et al.*, 2008). On the other hand, very few studies regarding rotifers culture have been conducted in Bangladesh, although this country is potentially rich for aquaculture.

Rotifers have specialized organ systems and a complete digestive tract that includes both a mouth and anus. As rotifers are microscopic animals, their diet must consist of matter small enough to fit through their tiny mouths during filter feeding. Rotifers are primarily omnivorous, but some species have been known to be cannibalistic. The diet of rotifers most commonly consists of dead or decomposing organic materials, as well as unicellular algae and other phytoplankton that are primary producers in aquatic communities. Such feeding habits make some rotifers primary consumers. Rotifers are in

turn prey to carnivorous secondary consumers, including shrimp and crabs. *Brachionus* feeds on microalgae, protozoa, bacteria and dead organic materials (Rezeq and James, 1987) in addition to artificial feeds. Diet is regarded as the most important criterion that could affect growth as well as nutritive quality of rotifers (Lubzens, 1987; Nhu, 2004).

Since large-scale algal production is relatively cheap, under field and laboratory conditions, various types of algae are routinely being produced for feeding planktonic rotifers (Groeneweg and Schluter, 1981). *Chlorella* is an excellent food for rotifer if supplemented with vitamin B_{12} (Hirayama *et al*., 1989) and some strain of *Chlorella* are known to absorb vitamin B_{12} from culture medium and store this vitamin in their cells (Maruyama and Hirayama, 1993). In the present study, we cultured *Chlorella ellipsoidea* in different media and used as food in powdered and fresh live form along with Backer's yeast in culture of the rotifer, *Brachionus calyciflorus*.

Materials and Methods

Experimental design

The culture experiment of rotifer was conducted in a balcony of a room at 2nd floor of the Fisheries Faculty Building of Bangladesh Agricultural University, facing the north on a steel shelf having 3 sides partially closed with a light coloured polyester cloth (Fig. 1). The experiment had three treatments with three replications i.e., treatment 1 (T1), in which the powdered dried *Chlorella ellipsoidea* was used as feed for rotifer, treatment 2 (T2), in which fresh cultured *C. ellipsoidea* were used as feed and treatment 3 (T3), in which baker's yeast was used as feed.

For supplying food to rotifers, the culture experiment of *C ellipsoidea* was also conducted at the same place where there is sufficient sun light for their growth (Figure 1). This experiment had also three treatments with four replications i.e., treatment 1 (T1), in which the expensive medium (inorganic), treatment 2 (T2), in which the inexpensive medium (pulse bran extract) and treatment 3 (T3), in which the inexpensive medium (soil extract) were used for *C. ellipsoidea* culture.

Fig. 1. Culture of *Chlorella ellipsoidea* and *Brachionus calyciflorus* on a steel shelf

Preparation of media for Chlorella ellipsoidea culture

Preparation of inorganic culture medium

Inorganic medium was prepared with the inoculation of stock solutions of 8 major (macro) nutrients and 6 minor (micro or trace) nutrients. Ten litre distilled water was taken in a 30 litre plastic bucket and stock solutions were added and mixed well in the bucket and stored in a 15 litre plastic container. Stock solutions were prepared in distilled water using different chemical compounds as major nutrients and trace elements (Table 1).

Table 1. Composition of inorganic algal growth medium (modified GBII algal growth medium of Stainer *et al.*, 1971)

Ingredients (compounds)		Concentration in stock solution	Inoculation in growth
Major nutrients	$NaNO_3$	75.0 g 500 ml^{-1}	100 ml 10 L^{-1}
	$MgSO_4.7H_2O$	15.0 g 200 ml^{-1}	10 ml 10 L^{-1}
	K_2HPO_4	15.0 g 200 ml^{-1}	10 ml 10 L^{-1}
	$CaCl_2.2H_2O$	15.0 g 200 ml^{-1}	10 ml 10 L^{-1}
	Na_2CO_3	15.0 g 200 ml^{-1}	10 ml 10 L^{-1}
	EDTA	15.0 g 200 ml^{-1}	10 ml 10 L^{-1}
	Ferric ammonium citrate	15.0 g 200 ml^{-1}	10 ml 10 L^{-1}
	Citric acid	15.0 g 200 ml^{-1}	10 ml 10 L^{-1}
Trace elements	$MnCl_2.4H_2O$	1.810 g L^{-1}	10 ml 10 L^{-1}
	$Na_2MoO_4.H_2O$	0.390 g L^{-1}	10 ml 10 L^{-1}
	$ZnSO_4.7H_2O$	0.220 g L^{-1}	10 ml 10 L^{-1}
	$CuSO_4.7H_2O$	0.079 g L^{-1}	10 ml 10 L^{-1}
	$CaSO_4.7H_2O$	0.049 g L^{-1}	10 ml 10 L^{-1}

Preparation of inexpensive pulse bran organic culture medium

Pulse bran (Maskalai bran, *Vinga mungo*) was mixed with 20 L tap water in plastic bucket. After one week 11g urea was added to each bucket. After four weeks partially decomposed pulse bran mixture was filtered through thin markin cloth and solid materials were discarded. Then after a week the supernatant was siphoned to another bucket and 2 g lime (CaO) per litre 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 algae culture medium.

Preparation of inexpensive soil extracts culture medium

Soil extract medium was prepared according to Rahman (2011). In brief, soil was collected from bottom of a nursery pond of Field Laboratory Complex of Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Textural class of the collected soil was "silty clay loam" which is one of the very fertile soils. After drying for 2 weeks, soil was crushed into powder to facilitate sieving. Soil was sieved through a small mesh sieve usually used to sieve rice powder for making cake. Then 2 kg soil was mixed with 5L tap water in a plastic bucket. Soil-water mixture was kept for 5 days and during this period mixture was stirred regularly for half an hour. Then soil-water mixture was kept in this condition for several days till the settling of soil particles at the bottom of the bucket. Then supernatant was collected and sterilized in an autoclave at 121°C temperature and 15 Ib/$inch^2$ pressure for 20 minutes. The soil extract was treated with commercial urea (5.0 g per litre) and TSP (2.5 g per litre) fertilizers.

Culture of C. ellipsoidea in prepared three media

The seeds of *Chlorella ellipsoidea* were collected from previous and continuous culture maintained in the laboratory of the Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh. Twenty percent seeds of *C. ellipsoidea* were used as inoculums in 200 ml culture medium taken in 1000 ml conical flask. Estimations of cell density of culture of *C. ellipsoidea* collected from twelve conical flasks were done daily by a Haemacytometer following the procedure of Rahman (1992).

Drying and making powder of cultured C. ellipsoidea

The frozen concentrated *C. ellipsoidea* after thawing was dried in a microwave oven (SHARP, Japan). Then it was powdered with a mortar and pestle. Then the powder was kept in a glass tube having stopper to use as feed for rotifer.

Culture of rotifers, Brachionus calyciflorus

Rotifers seed collection

The seeds of rotifer were collected from different ponds around the Fisheries Faculty Building, Bangladesh Agricultural University, Mymensingh through selective netting with plankton nets (mesh size 55 micrometer and 250 micrometer).

Preparation of stock culture

The seeds of rotifer were cultured in 4 plastic jars (of 5 litre capacity). Continuous aeration for 24 hrs by air pumps was arranged. Fresh cultured *Chlorella* was used as food for the rotifer cultured. Samplings were done regularly from each of the plastic jars for preservation (in 5% formalin) and daily analysis under a compound microscope using a special zooplankton counting cell to observe the animal and to find out culturable rotifer. At the time when the concentration of rotifer was high and the concentration of nauplii, protozoa etc. gradually decreased and finally vanished, then the whole culture was considered as stock culture.

Stocking of Brachionus calyciflorus

Brachionus calyciflorus was cultured in nine plastic jars of 3 litre capacity each containing 1 litre of water. Initial density of *B. calyciflorus* was 3 individuals/ml in each jar and it was taken from the stock culture of rotifer.

Feeding

In T1 powdered dried *C. ellipsoidea* was given daily as feed for rotifer at the rate of 0.1 g per litre of water, in T2 fresh cultured *C. ellipsoidea* was given as 40 ml of the concentration 2.5 x 10^6 cells/ml and in T3 baker's yeast was given daily at the rate of 0.15 g per litre. Before use in the experiments, the *C. ellipsoidea* powder and the baker's yeast were suspended in small amount of water and homogenized by hand mixing.

Aeration

Continuous aeration for 24 hours was arranged by aerators (SIGMA 4000 SW, Japan) connected by a narrow plastic pipe and air stone on one end of the pipe to maintain an adequate supply of oxygen in every jar, during rotifer culture experiment.

Estimation of B. calyciflorus densities

Determination of *Brachionus calyciflorus* densities were done daily by using a special zooplankton counting cell under a compound microscope.

Environmental factors

Various environmental factors such as light intensity (lux), sunshine periods (hrs), water temperature (°C), air temperature (°C), pH, dissolved oxygen (mg L^{-1}) were estimated regularly following standard methods during the culture periods of *Chlorella* and rotifers.

Statistical analysis

Values are expressed as means ± standard error of the mean (SEM). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to assess statistically significant differences among the different sampling days and different treatments. Statistical significance was set at $p < 0.01$. Statistical analyses were performed using SPSS Version 14.0 for Windows (SPSS Inc., Chicago, IL).

Results

Environmental factors

The results of environmental factors are shown in Table 2. Throughout the study period light intensity (lux), sunshine periods (hrs), water temperature (°C), air temperature (°C), pH, dissolved oxygen (mg L^{-1}) were within the productive ranges and showed no abrupt changes during the experimental period in all the treatments.

Table 2. Environmental factors (Means ± SEM; n=3) during the experimental culture periods

Parameters	Treatments		
	T1	T2	T3
Light intensity (Lux)	3366.17 ± 71.96	3366.17 ± 71.96	3370.08 ± 112.56
Sunshine period (hrs)	7.13 ± 0.34	7.13 ± 0.34	7.13 ± 0.34
Water temperature (^{0}C)	23.18 ± 0.42	23.48 ± 0.46	23.15 ± 0.45
Air temperature (^{0}C)	24.33 ± 0.48	24.41 ± 0.43	24.51 ± 0.42
pH	7.88 ± 0.14	7.87 ± 0.17	7.71 ± 0.12
Dissolved oxygen (mg L^{-1})	13.86 ± 1.39	13.42 ± 1.23	13.48 ±1.31

Cell densities of the Chlorella ellipsoidea

Cell densities (x10^6 cells ml^{-1}) of *Chlorella ellipsoidea* cultured in different media for a period of 13 days have been presented in Figures 2 and 3. The ranges of cell density of *C. ellipsoidea* were 2.345 to 10.685 (x10^6 cells ml^{-1}), 3.496 to 12.596 (x10^6 cells ml^{-1}) and 2.765 to 11.698 (x10^6 cells ml^{-1}) in T1, T2 and T3, respectively. Maximum cell densities of *C. ellipsoidea* was recorded in inexpensive pulse bran extract medium (T2) during the culture period.

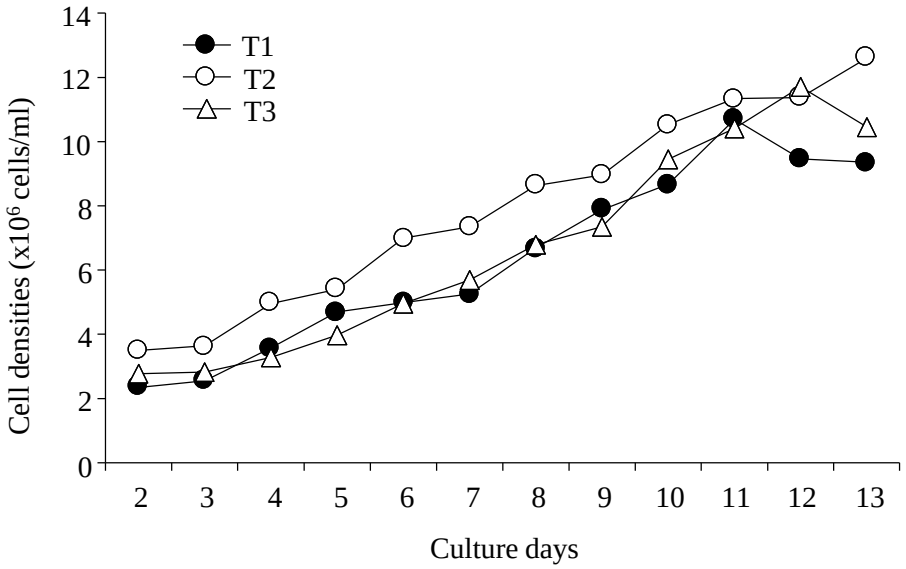

Fig. 2. Daily fluctuations of cell densities (x 10^6 cells ml^{-1}) of *C. ellipsoidea* in three media during the culture period of 13 days

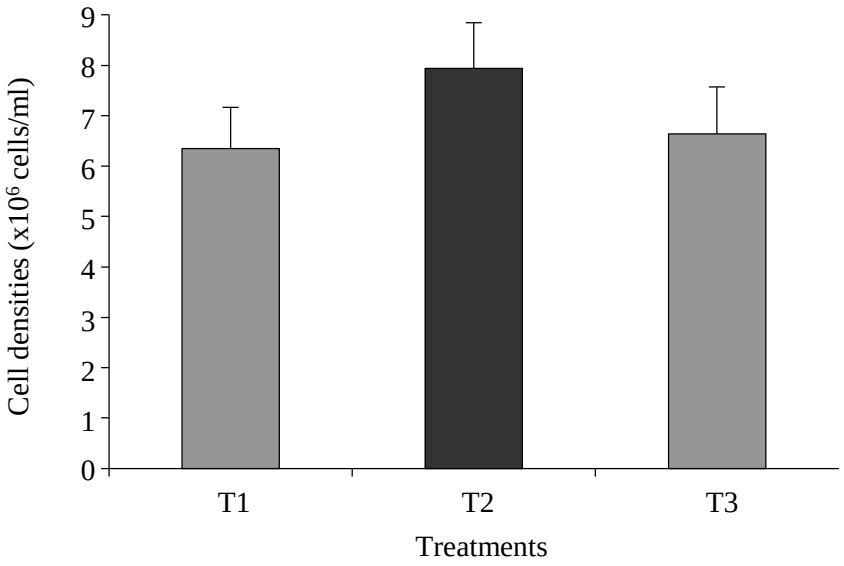

Fig. 3. Mean (Means ± SEM) cell densities (x 10^6 cells ml^{-1}) of *C. ellipsoidea* in three media during the culture period of 13 days

Population densities of rotifers, Brachionus calyciflorus

Population densities (x10^3 individuals L^{-1}) of *Brachionus calyciflorus* cultured in different treatment for a period of 10 days has presented in Figures 4 and 5. The ranges of population density of *B. calyciflorus* were 3 to 16 (x10^3 individuals L^{-1}), 3 to 48 (x10^3 individuals L^{-1}) and 3 to 20 (x10^3 individuals L^{-1}) in T1, T2 and T3, respectively. Mean population densities (means ± SEM) were significantly highest ($p<0.01$) in T2 (28.6 ± 4.64 (x10^3) individuals L^{-1}) compared to T1 (11.6 ± 1.24 (x10^3) individuals L^{-1}) and T3 (11.4 ± 1.82 (x10^3) individuals L^{-1}).

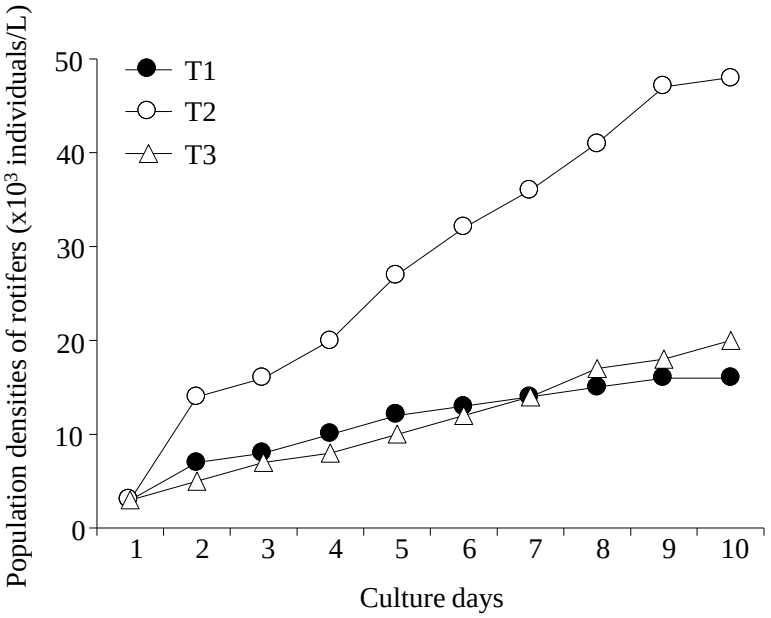

Fig. 4. Daily fluctuations of population densities (x 10^6 cells ml^{-1}) of *B. calyciflorus* in three treatments during the culture periods

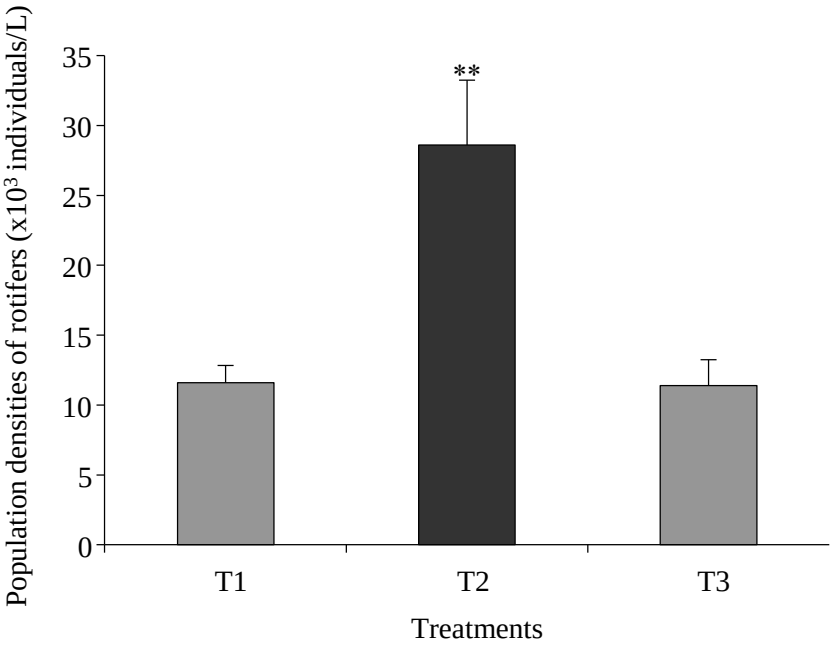

Fig. 5. Mean (Means ± SEM) population densities (x 10^6 cells ml^{-1}) of *B. calyciflorus* in three treatments during the culture periods. ** indicated significant difference at $p<0.01$

Discussion

The present study was conducted to evaluate the suitability of *Chlorella ellipsoidea* as food for the rotifer *Brachionus calyciflorus* production. We demonstrated the highest mean population density of *B. calyciflorus* in T2 where rotifer was fed on fresh cultured live *C. ellipsoidea* indicated that fresh cultured live *C. ellipsoidea* is the best food for production of the rotifer, *B. calyciflorus*.

In the present study, the ranges of cell densities of *C. ellipsoidea* were 2.345 to 12.596 (x10^6) cells ml^{-1} cultured in different culture media. The maximum cell density (12.596 x 10^6 cells ml^{-1}) was in inexpensive pulse bran extract medium on 13th day of culture (Fig. 2). The range of cell density of *C. ellipsoidea* that found by Hossain (1996) was 0.09 to 3.63 (x 10^6) cells ml^{-1}, which was lower than that of the present experiment. On the other hand, James *et al.* (1998) observed that the range of cell density was 20 x 10^6 to 80 x 10^6 cells ml^{-1}, which was much higher than that of the present experiment. These variations of production of *Chlorella* might be due to culture periods, quality of culture medium and environmental conditions.

Live algae supported the best growth of rotifers (Hirayama and Nakamura, 1976). Live *Chlorella* is one of the most widely used foods for culturing planktonic rotifers (Pourriot and Rougier, 1997). The mean value of *B. calyciflorus* density fed on fresh cultured *Chlorella ellipsoidea* under T2 of the present experiment was 28.6 ± 4.64 (x10^3) individuals L^{-1}, which is more or less similar to Awaiss *et al.* (1992), who reported that the production of *B. calyciflorus* fed on live *Chlorella* was on average 31.5 ± 3.5 (x10^3) individuals L^{-1}. The mean population density of *B. calyciflorus* fed on powdered dried *C. ellipsoidea* under T1 was 11.6 ± 1.24 (x10^3) individuals L^{-1}, which strongly agrees with Lucia *et al.* (2001) who found the population density of *B. calyciflorus* cultured feeding with heat-killed *Chlorella* ranged from 6 ± 1 to 26 ± 6 (x10^3) individuals L^{-1}. Hirayama and Nakamura (1976) found 400 individuals ml^{-1} during mass culture of *B. plicatilis* feeding with dry powder of *Chlorella* cultured for 41 days, which is higher than that of the present study, might be due to longer days of culture period. The mean value of *B. calyciflorus* fed on baker's yeast under T3 of the present experiment was 11.4 ± 1.82 (x10^3) individuals L^{-1}. Rahman *et al.* (1993) found that the mean values of *B. calyciflorus* fed on baker's yeast was 24.17 ± 5.40 (x 10^3) individuals L^{-1} which is much higher than that of the present study.

It is reflected that the mean population density of *B. calyciflorus* was higher under T2 where rotifer was fed on fresh cultured live *C. ellipsoidea* and the mean population density of *B. calyciflorus* under T3 where rotifer was fed with baker's yeast was lower than those of T1 and T2. This indicates that fresh cultured live *C. ellipsoidea* is the best food for the rotifer, *B. calyciflorus* and dried powder *C. ellipsoidea* is also better than baker's yeast as food for the rotifers. Although a diet of baker's yeast alone was not comparable to that of *C. ellipsoidea,* it can be effectively used at low concentration to supplement algal requirements in rotifer culture system (Sarma *et al*., 1997). It could be possible that dried and preserved *C. ellipsoidea* will be used when there is no live *C. ellipsoidea* to maintain rotifer cultures.

The environmental factors during *C. ellipsoidea* culture and rotifer, *B. calyciflorus* culture were found to vary within suitable ranges (Table 2). Within limit productive ranges of such water quality parameters have been observed by a number of authors (Chowdhury *et al*., 2008; Rahman *et al*., 2012; Talukdar *et al*., 2012; Siddika *et al*., 2012) for the proper growth and production of phytoplankton and zooplankton in natural conditions in the aquaculture ponds which are almost similar to those of the present study.

In conclusion, culture experiment of the rotifer, *B. calyciflorus* was done feeding with powdered dried *C. ellipsoidea*, fresh cultured live *C. ellipsoidea* and baker's yeast. The environmental factors during *C. ellipsoidea* culture and the rotifer, *B. calyciflorus* culture were found to vary within suitable ranges. The mean population densities of *B. calyciflorus* under T2 were significantly higher than those of T1 and T3. The results of present study revealed that fresh cultured live *C. ellipsoidea* is the best food for *B. calyciflorus* production and dried powdered *C. ellipsoidea* is better than baker's yeast as food for the rotifer.

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