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Evaluation of Yeast *Saccharomyces cerevisiae* and Algae *Chlorella vulgaris* as Diet for Rotifer *Brachionus calyciflorus*

B. Khatun[1]*, R. Rahman[2] and M. S. Rahman[1]

[1]*Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh, Bangladesh*
[2]*Abandoned Property Management board, Dhaka, Bangladesh*

**Corresponding author and Email: bornalik@yahoo.com*

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Abstract

A study was conducted to evaluate the effect of yeast *Saccharomyces cerevisiae* and algae *Chlorella vulgaris* on the production of rotifer *Brachionus calyciflorus*. The *Brachionus calyciflorus* were cultured using four different types of diets: fresh *Chlorella vulgaris* (T_1), yeast with fresh *Chlorella vulgaris* (T_2), powder *Chlorella vulgaris* with fresh *Chlorella vulgaris* (T_3) and yeast with powder *Chlorella vulgaris* (T_4). We compared these diets to find out their potentiality on the population growth of *Brachionus calyciflorus*. The rotifers were cultured under natural environmental condition with continuous artificial aeration. Initial density of *Brachionus calyciflorus* were 8 individuals ml^{-1} (ind. ml^{-1}). Water temperature and pH were measured during the culture period. Among the four different diets the fresh *Chlorella vulgaris* (T_1) and it's yeast combination (T_2) gave satisfactory production of rotifer 73±9 and 67±8 ind. ml^{-1}, respectively. However, processing of fresh *Chlorella vulgaris* to produce powder form declined the production ability of rotifer. The diet sample T_3 and T_4 containing powdered *Chlorella vulgaris* produced 48±9 and 25±6 ind. ml^{-1}, respectively. So, the diet in combination of yeast and fresh *Chlorella* is suggested for the sustainable production of the rotifer *Brachionus calyciflorus*.

Keywords: Rotifer, *Brachionus calyciflorus*, *Chlorella vulgaris*, yeast, diet

1. Introduction

Worldwide expansion of hatcheries with the remarkable developments in larval rearing technology demands suitable culture technique of rotifers as important larval food. Freshwater aquacultures, in particular the hatchery industry, are confronting the major obstruction for suitable larval food for larviculture. The successes of any hatchery system virtually rely on the availability of suitable live food organisms (Dhert *et al*., 2001). Rotifers are the most vital live food organisms used for rearing of fish larvae due to their size, nutritional value, and behavior (Arimoro, 2006). In addition, they can tolerate high range of temperatures and pH (Ludwig, 1993). *Brachionus calyciflorus* is a suitable live food for larval feeding and can be cultured, enriched with nutrients and used along with other freshwater rotifers (Rufchaie *et al*., 2012).

Although a number of techniques have been developed for the mass production of rotifers (Dhert *et al*., 2001), problems concerning unexpected stagnant growth and the sudden collapse of the rotifer mass culture are yet to be solved (Yoshinaga *et al*., 2001). On the other hand, various studies have shown that the

considerable factors influencing the rotifer culture are food supply and concentration of species (Sarma *et al*., 2001; Lucia-Pavon-Meza *et al*., 2005). Fresh algae, frozen or hit killed algae, algae powder and baker's yeast, are some of the food sources that have been exploited for the culture of rotifers (Lucia-Pavon *et al*., 2001; Ashraf *et al*., 2010). Besides, a medium which contained a mixture of yeast, starch and albumen was applied for the growth of rotifers (Sulehria *et al*., 2010).

Chlorella was widely utilized in different concentrations for three species of rotifers; *Brachionus angularis, B. calyciflorus and B. plicatilis* (Pourriot and Rougier, 1997). Plainly live green algae is the best food for the growth of *Brachionus calyciflorus* (Lucia-Pavon *et al*., 2001) but the mixture of algae and yeast are more effective than algae alone (Ashraf *et al*., 2010; Pena-Aguado *et al*., 2005). On the other hand, baker's yeast are acceptable as diet for *Brachionus* in short time because of its incomplete nutrient for rotifer culture (Hirayama *et al*., 1989; Watanabe *et al*., 1983) but only yeast is not suitable for *Brachionus calyciflorus* (Sarma *et al*., 2001). However, algae were gradually replaced by yeast for the production of rotifer to reduce dependency on labor intensive live food (Asraf *et al*., 2010).

Rotifer production is significantly influenced by the quantity and quality of food given (Rajendiran and Subramanian, 2007). Although dried *Chlorella* powder was considered as effective food for the rotifer *Brachionus plicatilis* (Hirayama and Nakamura, 1976) but frozen *Chlorella* is not suitable for rotifer (Lucia *et al*., 2001). On the other hand, Mostary *et al*. (2007) reported that powder dried *Chlorella* had significant effect on the population density of *B. angularis* and this was suggested as supplementary feed for *B. plicatilis* (Mostary *et al*., 2010). The cost-effective biomass production of rotifers relies on the use of a cheap food source (Dhert *et al*., 2001). Although *B. calyciflorus* might have good prospect in cost-effective biomass production but little is known about the culture, nutrition, and population dynamics of freshwater rotifer, *B. calyciflorus* (Rajendiran and Subramanian, 2007).

Not much attention has been given on culture of rotifer *B. calyciflorus* particularly, in the local environment in Bangladesh. Supply of good quality and sufficient micro algae as the sole food for rotifer culture is usually considered not only labor-intensive but also expensive. Therefore, concerning these facts, to make our hatcheries a successful endeavor the aims of the current study were designed to evaluate the effect of fresh *Chlorella vulgaris*, yeast *Saccharomyces cerevisiae* and powder *Chlorella vulgaris* as combined diet of rotifer *B. calyciflorus* production.

2. Materials and Methods

2.1. Materials

Pulse bran *Vigna mungo* was purchased from the market. Seeds of algae *Chlorella vulgaris* were collected from previous stock culture maintained under natural environmental conditions at Bangladesh Agricultural University. The seeds of rotifer *Brachionus calyciflorus* were collected from different ponds. Yeast was also purchased from the local market. *Chlorella* powder was prepared from the cultured fresh *Chlorella vulgaris* during the experimental period.

2.2. Methods

2.2.1. Preparation of organic medium for *Chlorella* culture

Three plastic buckets of 30 liter capacity were used to prepare the medium of pulse bran. First, pulse bran *Vigna mungo* was mixed at the rate of 700 g in 20 liter tap water in plastic buckets. Next week, 11 g urea was added to each bucket. After four weeks, partially decomposed pulse bran mixture was filtered through thin cloth and solid materials were discarded. The following week, the supernatant was siphoned to another bucket and 1.5 g CaO per liter of medium was mixed to make it clear and pH was adjusted to 7 adding required amount of H_2SO_4. After a week, the clear supernatant was siphoned again to

another bucket and this almost clear solution was ready as algae culture medium. The prepared medium was sterilized in an autoclave at 121°C temperature under 15 1b/cm^2 pressures for 15-20 minutes.

2.2.2. Culture and powder formation of *Chlorella vulgaris*

The productivity of algae depends on some major factors, e.g. nutrition, light, temperature dissolved oxygen and pH. *Chlorella* was cultured in batches. The duration of culture in each batch was 21 days. For *Chlorella* culture, 18 conical flasks of 500 ml capacity were used. All the conical flasks were arbitrarily numbered as 1-18 for the convenience of the research. In each conical flask, 200ml of sterilized pulse bran medium and 50 ml *Chlorella vulgaris* seeds (1.2×10^6 cells ml^{-1}) were used as inoculums. So, the initial density of *Chlorella vulgaris* in the conical flasks was 0.24×10^6 cells ml^{-1}. The culture experiment was done in natural sunlight and temperature condition in a north facing balcony of a room. The water temperature range was 26 to 30^{0}C. The mean sunshine period and rainfall were 7.18 ± 3.49 hours and 0.0 mm, respectively. Cell densities of *Chlorella vulgaris* collected from conical flasks were estimated daily using hemocytometer. Cell densities of *Chlorella vulgaris* were calculated using the following formula (Rahman, 1992):

Cells ml^{-1} = Average number of cells in 1 cubic mm × 1000

Cultured *Chlorella vulgaris* was centrifuged (2000 rpm) for 5 minutes to collect the dense sample of *Chlorella*, which was preserved in the freeze. The frozen *Chlorella vulgaris* after thawing was dried in a microwave oven (SHARP, Japan) and powdered with a mortar and pestle. The powdered *Chlorella* was kept in a hygienic plastic container for use as feed for rotifer.

2.2.3. Stock culture of rotifer (*Brachionus calyciflorus*)

Seeds of *Brachionus calyciflorus* were cultured in four plastic jars of 5 liter capacity for one month, with continuous aeration for 24 hrs by air pumps. To achieve pure culture of the rotifer 'Basudine' was applied at the rate of 1.5 mg l^{-1} (Arimoro and Ofojekwu, 2004). Fresh cultured *Chlorella vulgaris* was used as food. Samples were taken every couple of days from each plastic jar for preservation (in 70% ethanol) and analyzed under a compound microscope using Sedgwick-Rafter (S-R) counting cell until the concentration of rotifer was high. When the concentration of rotifer was high the whole culture was considered as stock culture.

2.2.4. Experimental Culture of rotifer (*Brachionus calyciflorus*)

Brachionus calyciflorus were cultured in 16 plastic jars of 3 liter capacity, each of them containing 1 liter of tap water. Initial density of *Brachionus calyciflorus* was 8 ind. ml^{-1} in each jar. These seeds were taken from the stock culture of rotifer. The duration of culture was 14 days. In this experiment, four treatments were used. Among the 16 plastic jars the jar numbers 1a, 1b, 1c, 1d were under T_1. Jar numbers 2a, 2b, 2c, 2d were under T_2. The jar numbers 3a, 3b, 3c, 3d were under T_3 and the jar numbers 4a, 4b, 4c, 4d were under T_4. Fresh *Chlorella vulgaris* was given at the rate of 1.2×10^6 cells ml^{-1} of water when used in combination with yeast and powder *Chlorella vulgaris*. Yeast was given at the rate of 0.08 mg ml^{-1} of water and powder *Chlorella vulgaris* was given at the rate of 0.05 mg ml^{-1} of water. Layout of the rotifer culture has been shown in Table 1. Before use in the experiments, the *Chlorella* powder and yeast were well mixed with water separately. Then each mixture was kept in a test tube having screw cap stopper for use as feed for rotifer.

Continuous artificial aeration for 24 hours was arranged by two aerators (SIGMA SW, Japan). The aerators were connected by a narrow plastic pipe to maintain an adequate supply of oxygen in each jar during culture. Water temperature and pH were recorded daily. Water temperature range during rotifer culture was 24.5 to 30^{0}C with air supply. pH was near about 7 but with a range of 6.8 to 7.3.

Table 1. Experimental layout of rotifer (*Brachionus calyciflorus*) culture

Treatments	diets for rotifer	Replications
T_1 (control)	fresh *Chlorella vulgaris* (1.6×10^6 cells ml^{-1})	4
T_2	yeast (0.08mg ml^{-1}) with fresh *Chlorella vulgaris* (1.2×10^6 cells ml^{-1})	4
T_3	powder *Chlorella vulgaris* (0.05mg ml^{-1}) with fresh *Chlorella vulgaris* (1.2×10^6 cells ml^{-1})	4
T_4	yeast (0.08mg ml^{-1}) with powder *Chlorella vulgaris* (0.05 mg ml^{-1})	4

Determination of *Brachionus calyciflorus* density was done daily by using Sedgwick-Rafter (S-R) counting cell which was 50 mm long, 20 mm wide and 1 mm deep. Before filling the S-R cell with sample, the cover glasses were diagonally placed across the cell and then samples were transferred with a large bore pipette to avoid the air bubbles in the cell covers. Then rotifers on the bottom of the S-R cell were enumerated by compound microscope (40 x magnifications). Number of rotifer in the S-R cell was counted using the following formula (APHA, 1976; Shil *et al*., 2013):

$$No./ml = \frac{C \times 1000\ mm^3}{L \times D \times W \times S}$$

Where,
C = Number of Organisms Counted
L = length of each strip (S-R cell length) in mm
D = depth of a strip in mm
W = width of each strip (mm)
S = number of strips counted.

The number of cells per milliliter was multiplied by the dilution factor to adjust the number of organisms.

The data were analyzed by the statistical analysis tools (Anova- single Factor and t- Test: Paired Two Sample for Means) of Microsoft Excel 2010.

3. Results and Discussion

3.1. Culture of Chlorella vulgaris

The maximum cell density of *Chlorella vulgaris* was found after 13 days (Fig. 1). The produced cell density was 4.47×10^6 cells ml^{-1}, which is a good agreement with other reported findings (Mostary *et al*., 2010). They also found their highest production of 4.489×10^6 cells ml^{-1} after 13 days of culture in the same culture media. Compared to previous studies, our applied culture media and environmental factors are widely used application for satisfactory production of *Chlorella* sp. (Chowdhury *et al*., 1995 and Sharma *et al*., 2012).

3.2. Culture of rotifer B. calyciflorus

The maximum production of *B. calyciflorus* using fresh *Chlorella vulgaris*, the control diet without any treatment (T_1), was 73±9 ind. ml^{-1}. Very similar results were reported by other workers (Sarma *et al*., 2001; Pena-Aguado *et al*., 2005). For example Sarma *et al*. (2001) found 77±12 ind. ml^{-1} rotifer production by using $1x10^6$ cells ml^{-1} concentration of *Chlorella vulgaris* as food. In T_2 using the combined diet of yeast with fresh *Chlorella vulgaris* the maximum number of rotifer production was 67±8 ind. ml^{-1}. Similar result was found by Pena-Aguado *et al*. (2005). In T_3 using the combined diet of powder *Chlorella vulgaris* with fresh *Chlorella vulgaris* the highest no of *B. calyciflorus* production was 48±9 ind. ml^{-1}. It has been reported that using powder *Chlorella* for the culture of *B. anguilaris* the maximum production was 50 ind. ml^{-1} (Mostary *et al*., 2007). In T_4 using the combined diet of yeast with powder *Chlorella vulgaris* the maximum number of rotifer production was 25±6 ind. ml^{-1} that was very poor production.

The trends of population densities of *B. calyciflorus* under four treatments are presented

in Fig. 2. The peak rotifer production was noticed at the eighth day for T_1 and T_3 followed with gradual decline. The negative effect on rotifer production due to toxin related to the high density of rotifer is evident (Shertzer *et al*., 2002). Therefore, the researcher sometimes completed their harvest after only 8 days of culture (Rajendiran and Subramanian, 2007). However, giving yeast in the diets increased the time to reach at the peak point of rotifer production. Both of our experimental diets with yeast combination (T_2 and T_4) have given the maximum yield after 10 days of culture (Fig. 2). The increased time to get maximum culture population of rotifer due to yeast effect was also by Arimoro and Ofojekwu (2004).

According to statistical analysis (ANOVA) as $F > F$ crit, we rejected the null hypothesis (Table 2). So, there are significant differences of population density of rotifer *B.* calyciflorus under four treatments.

3.3. Comparison of the diets

Fig. 2 shows that among the four different treatments, T_1 (fresh *Chlorella vulgaris*) produced the maximum number of rotifer *B. calyciflorus* 73±9 ind. ml^{-1} than the other three treatments. Several literatures showed that fresh *Chlorella* give better yield of rotifer than other individual diet (Sarma *et al*., 2001; Lucia-Pavon *et al*., 2001). As *B. calyciflorus* is filter feeding rotifer, the particle size of *Chlorella* is suitable for their mouth opening and it has good nutritional quality (Rajendiran and Subramanian, 2007; Dhert *et al*., 2001). T_4 (yeast with Powder *Chlorella vulgaris*) did not show the satisfactory result. T_3 (powder *Chlorella vulgaris* with fresh *Chlorella vulgaris*) showed better result than T_4 but the production rate of rotifer by this diet is much lower than T_1 and T_2. T_2 (yeast with fresh *Chlorella vulgaris*) gave production close to T_1 (fresh *Chlorella vulgaris*). There was no significant difference between T_1 and T_2 ($P>0.05$).

It has been agreed in several literature that *Chlorella* alone and it's combination with yeast gives better result for population growth of *B. calyciflorus* (Sarma *et al*., 2001; Arimoro and Ofojekwu, 2004; Asraf *et al*., 2010; Pena-Aguado *et al.*, 2005). In this study comparatively higher production of rotifer from the diet in combination of yeast (T_2) than powder *Chlorella vulgaris* (T_3) with fresh *Chlorella vulgaris* is probably an indicator that yeast is more effective than powder *Chlorella* as combined food with fresh *Chlorella*.

The declined production ability of fresh *Chlorella* due to processing (heat-killed, frozen etc.) is articulated in the literature (Lucia-Pavon *et al*., 2001). The reason may be that powder *Chlorella* is difficult to digest for the test species and the degradation of nutritional quality. Moreover, powder *Chlorella* is expensive than yeast and to produce *Chlorella* powder there are some limitations regarding suitable time and workforce. On the other hand, the microorganisms associated with yeast may be beneficial for rotifer. Though only yeast is not suitable for rotifer culture (Sarma *et al*., 2001) but the diet in combination of yeast and fresh *Chlorella* can be suitable and cost effective for the culture of rotifer *B. calyciflorus* and better than other combinations (Asraf *et al*., 2010; Pena-Aguado *et al*., 2005).

3.4. Environmental factors

Water temperature range during our rotifer culture was within the optimum range of *B. calyciflorus* culture temperature (Arimoro, 2006; Park *et al*., 2001). It can be seen in (Fig. 3) that the temperature fluctuation during the culture period was between 24.5- 30^0C. In this study the pH range was 6.8 to 7.3 (Fig. 4). This pH level was also within the optimum range of rotifer culture (Park *et al*., 2001; Lucia *et al*., 2001). It should be noticed that the pH and temperature of the water in this study were not controlled but only recorded during the culture period.

Table 2. ANOVA: Single Factor analysis for the population density of rotifer under four different treatments

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	6797.353125	3	2265.784375	8.318416416	0.000114969	2.769430949
Within Groups	15253.375	56	272.3816964			
Total	22050.72813	59				

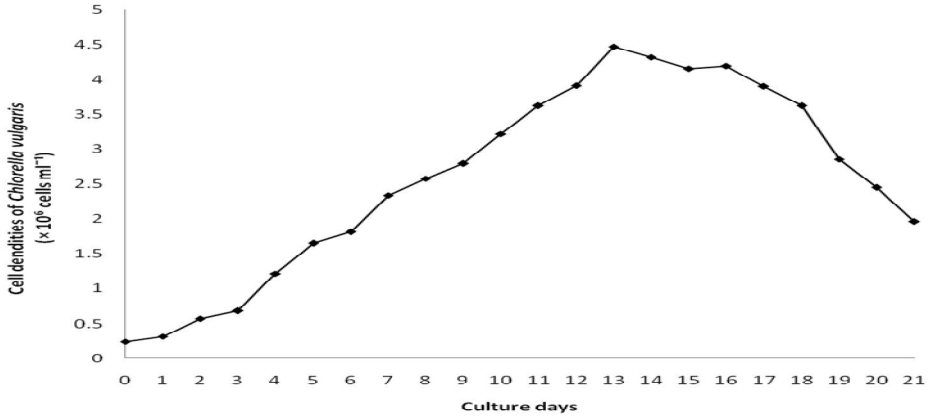

Fig. 1. Cell densities of *Chlorella vulgaris* ($\times 10^6$ cells ml^{-1}) during the culture period

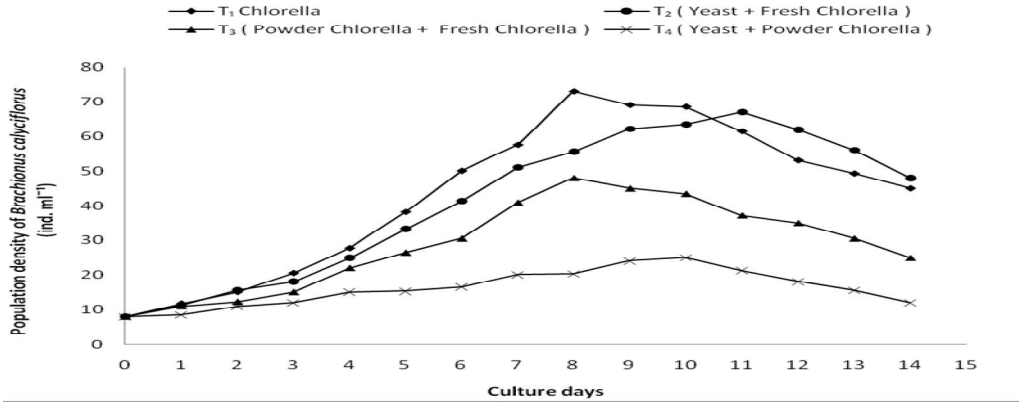

Fig. 2. Variations of population densities of *B. calyciflorus* (ind. ml^{-1}) cultured under T_1 (control), T_2, T_3 and T_4

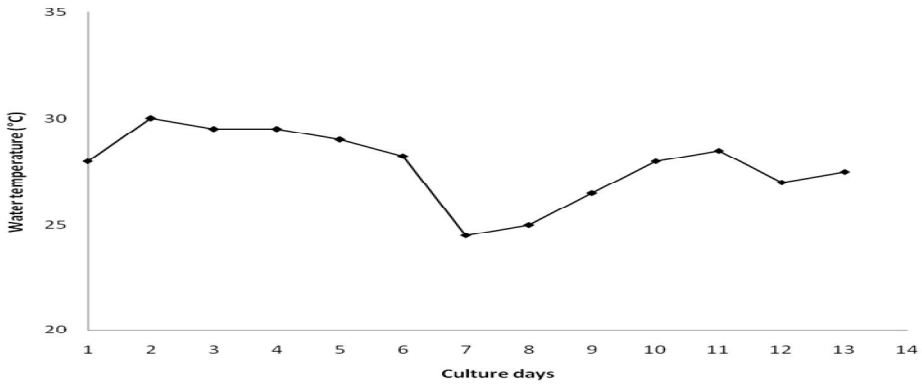

Fig. 3. Daily changes of water temperature (^{0}C) during the culture period of rotifer *B. calyciflorus*

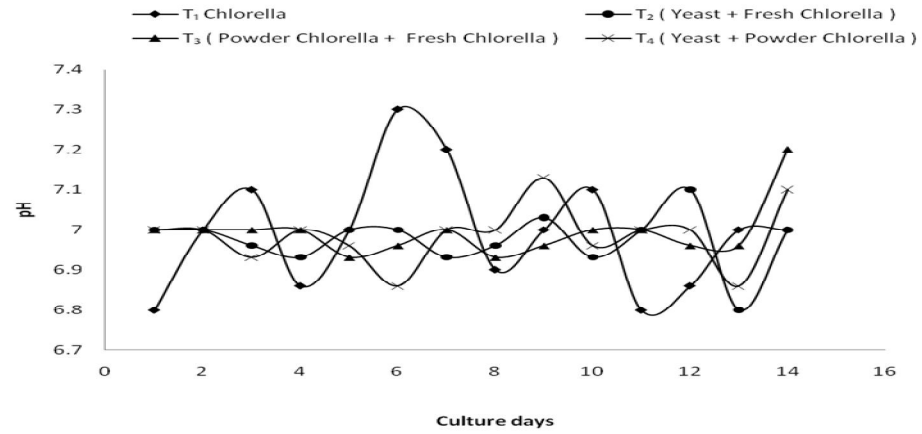

Fig. 4. Daily changes of pH during culture of *B. calyciflorus* under four different treatments

4. Summary and Conclusions

This study was carried out in light of getting an easy and cheaper way to support rotifer culture. The effects of addition of yeast and powder *Chlorella* with fresh *Chlorella* on production of rotifer were assessed. Those combined diets as well as fresh *Chlorella* alone were supplied to culture rotifer and the effects on production were measured by microscopic analysis. The rotifer culture was carried out under natural environmental conditions. It was found that yeast is more effective than powder *Chlorella* when used in combination with fresh *Chlorella* for culture of rotifer *Brachionus calyciflorus*. It is recommended that the improved rearing conditions will be able to give good rotifer production using the diet in combination of yeast

and fresh *Chlorella*. This would probably reduce the maintenance costs for algae and reduce the overall production costs of rotifer *B.* calyciflorus.

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