

Mass Production of *Moina*, *Moina brachiata* with microalgal species *Chlorella vulgaris* as feed and preservation of its dormant cysts

V. Rani*, S. Palanichamy** and N. Neethiselvan***

* Assistant Professor, TANUVAS-RRC, Pudukkottai- 622 004. E-mail: raniradha06@yahoo.co.in

** Technical officer, Mandapam Regional Centre of Central Marine Fisheries Research, Institute, Mandapam camp, Ramanathapuram District, Tamilnadu.

*** Associate Professor, Veterinary University Training and Research Centre, Nanjikkottai Road, Thanjavur-6. Tamil Nadu Veterinary and Animal Sciences University, Regional Research Centre, Industrial Estate PO, Pudukkottai - 622 004. Tamilnadu. India.

(Accepted for publication - 31st August, 2008)

Abstract

The freshwater microalgae *Chlorella vulgaris* was used as food for the large scale production of *Moina brachiata* (Cladocera). A maximum cell concentration of 28.5million cells/ml of *Chlorella vulgaris* within 7-10 days of inoculam was obtained using commercial low cost fertilizers of groundnut oil cake (250gm) along with traces of urea (10gm) and super phosphate (5gm)/tonne. The moina concentration after a week of introduction into mass culture tank reached 15,000 –19,000 no/lit. The dormant cysts of moina produced by manipulating the environmental culture conditions were collected and the preservation technique was perfected to store the cysts for about 2 years.

Key words : Commercial fertilizers, *Moina*, Preservation of dormant cysts.

Introduction

The microalgae are known to have a wide spread distribution in the oceans, lagoons, freshwater lakes and rivers. Some species of these algae which grow well in mass culture under controlled conditions have been successfully used as food for rearing larval stages of zooplankton and commercially important crustaceans, molluscs and finfishes. Microalgae used in aquaculture should

provide essential nutrients for animal growth and development. The type of microalgae for mass culture is chosen according to the specific nutritional requirements of the animals to be fed. The growth and quality of a particular species of microalgae can affect the success or failure of aquaculture industry.

In this investigation the freshwater microalgal species *Chlorella vulgaris* which is known to multiply rapidly in a short time under controlled

*Corresponding author.

conditions is used as food source to produce moina in large scale. Successful hatchery production of crustacean post larvae and fish fry depends on the availability of suitable feeds like artemia nauplii, copepods, mysids and moina.

Among these, *Moina* spp are known to have considerable economic importance as they form cheap source of live food for fishes and crustaceans living in wide range of habitats. The high reproductive rate, short generation period, high nutritive value etc, are the favourable characteristics of *Moina brachiata*, for its mass culture under controlled conditions. The *Moina brachiata* is an ideal food for the post larval stages of crustacean and fry of finfishes and as an important component of food chain in freshwater and brackishwater ecosystems. The resting eggs or the dormant cysts of moina are the product of sexual reproduction formed during unfavourable environmental conditions. The resting eggs could be collected from the bottom of the culture tanks along with sediments, cleaned, dried under shade and stored in different environmental conditions for future use. The cultured moina can be fed by siphoning drop by drop or in frozen condition in the marine larval rearing tanks (Dripping method).

The present study aims for the mass culture of freshwater microalgae *C. vulgaris* to produce moina and preserve its dormant cysts for longer period as the stock. The long term storiation of cysts will help to reduce the maintenance cost. The experiments were conducted at Mandapam Camp, CMFRI, for a period of about 2^{1/2} years during 09.11.1999 - 20.04.2002 and the results of the experiments are presented in this paper.

Materials and Methods

The material *Moina brachiata* was isolated from the freshwater pools situated in 'Pirappan

Valasai' near Uchipulzi at Ramanatl district, Tamil Nadu. The pure stock cul raised from ten parthenogenetic females. 42,000 numbers of moina were obtained v days from a single female.

The filtered freshwater was pumped i one tonne capacity tanks (Tank A and T and each tank was fertilized with 250 groundnut oil cake, 10 gm of urea and 5 super phosphate/tonne of water. Vigorous a was provided from the start of the culture. culture of 50 l *Chlorella vulgaris* was inocul each culture tank on the same day. The ino cell concentration was 20.8 million cells/ml ar initial cell concentration of the culture tanl 1.2 million cells/ml. On the second da fertilization, as the water became light green parthenogenetic female moina was introduce the rate of 0.8 no/ml in tank 'A'.

In tank 'B' moina was not introduced to s the growth of *Chlorella vulgaris* and the concentration was taken in an alternate days us haemocytometer. The environmental parameters the culture tanks were monitored in alternat days. The temperature ranged between 28 a 34°C, pH 6.75 and 9.75 and dissolved oxygen 1. and 6.85 ml/l. The ammonia content was 0 0.5mg/l.

The environmental parameters such as hig stocking density, less feeding rate and low oxygen level were maintained in the culture system for the production of dormant cysts.

Batch culture method-

Batch culture experiments of moina were conducted in one tonne circular FRP tanks which were introduced with *Chlorella vulgaris* as the diet. The duration of the experiment was 10 days and the cysts were harvested from the bottom of the

culture tank along with sediments.

Semi-Continuous culture method-

The experiment was conducted in two one tonne tanks. The *Chlorella vulgaris* was maintained separately for the exchange of medium in the experimental tanks. 20-30% of the culture medium was exchanged with *Chlorella* culture in 2-3 day intervals to readjust the moina concentration of 10,000-15,000/l and the less feeding level. The culture period was 16-18 days.

Preservation of cysts-

Various preservation methods were tried for the produced dormant cysts.

1. The cysts harvested along with sediments were dried in hot air oven under a constant temperature of 30°C. The cysts were then stored in glass tubes at temperature of 27-30°C.

2. Some dried cysts were stored in saline water of 10, 20 and 30 ppt at room temperature.

3. Another set of cysts were stored in closed dark glass container (covered with black carbon sheet) in refrigerator at 3-5°C and in the temperature of 27-30°C.

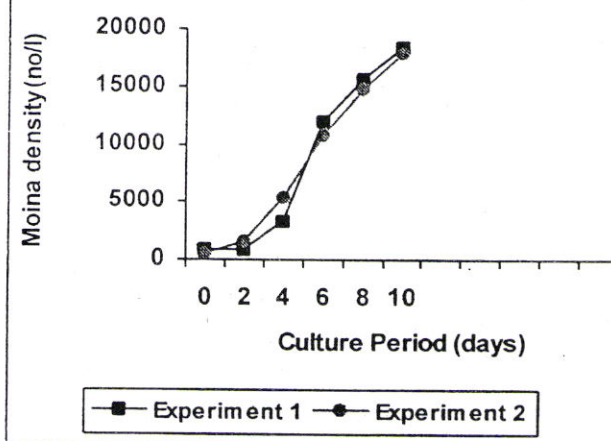
Results and Discussion

Tank A

The moina was found to feed on *Chlorella vulgaris* which showed growth utilizing fertilizers and natural sunlight. In the moina culture tanks, a concentration of 17, 869/l and 18, 436/l of moina was obtained within 7-9 days of culture period from the initial concentration of 500/l and 800/l in experiment 1 and 2 respectively (Fig.1).

At this stage, the moina was harvested by siphoning 1/3 of the water through 120 micron bolting silk cloth and replaced with freshwater containing proportionate amount of fertilizers or

Fig.1. Culture of Moina using *C. vulgaris* as microalgal feed



cultured *Chlorella* with the cell density of 20-25 million cells/ml.

The harvesting was done in the exponential growth phase by skimming the surface water with a plankton net during morning and evening.

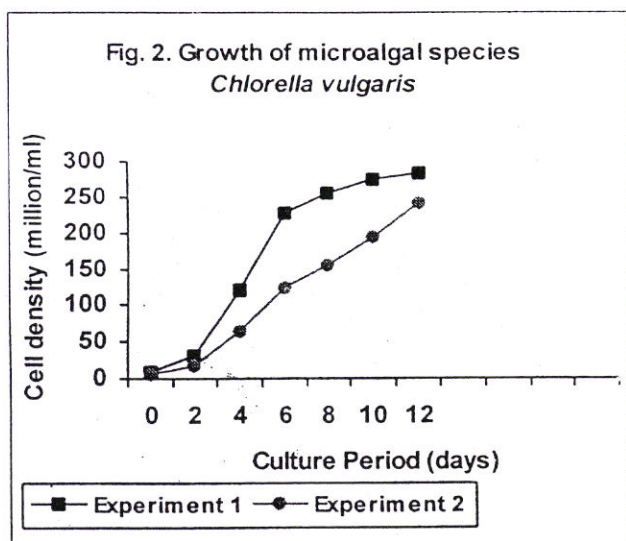
Tank B

In this tank moina was not introduced to study the growth of *Chlorella vulgaris*. The maximum concentration of *C.vulgaris* obtained was 28.5 and 24.2million cells/ml within 7-10 days of culture period from the initial concentration of 4.64 and 5.56 lakh cell/ml (Fig.2).

Production and preservation of cyst

In batch culture method, the number of cysts harvested in the culture tank along with sediments was 1.5 to 2.0 no/cm². In the case of semi-continuous culture method, the number of cysts harvested was 6.5-7.0/ cm².

1. The hatching rate of cysts preserved in room temperature (light and dark) was 10-20 % for the first 6 months and then it gradually reduced depending upon the period of preservation. In this method, no hatching was observed after 9 months of storage.



2. The hatching rate of cysts preserved in saline water was about 10% for the first 3 months and became nil after 6 months.

3. The hatching rate of cysts preserved in dark container at 3-5°C was 20-27.3 % up to 18 months, 15-20.5% up to 21 months and .5-10 % up to 29 months of preservation.

The cysts will hatch out within 24-36 hours of incubation in freshwater provided with vigorous aeration and light.

The lipid content of the green microalgae *C. vulgaris* (14.22%) is almost double than the content of diatoms *Skeletonema costatum* and *Chaetoceros calcitrans* (7% and 10% respectively) and almost equal to the lipid content of *Isochrysis galbana* (21%) Jai Sankar Ojha (2000). So it is clear that the moina fed with *Chlorella vulgaris* may contain the required amount of fatty acids essential for the growth of predator larvae. In commercial crustacean and finfish hatcheries, the artemia nauplii are normally fed to the post larvae and fry of finfishes. The cysts of artemia have to be imported and are also costlier. Moina cultured using *Chlorella vulgaris* as feed is not only economical but could also be a better substitute for artemia

nauplii to fulfill the nutritional requirements of predator larvae. In this study, to avoid over expenditure in the large scale production of *C. vulgaris*, the commercial grade low cost fertilizers of groundnut oil cake, urea and super phosphate were used and the maximum concentration of 28.5 million cells/ml were obtained. Dutta (2007) the total number of individual moina was $14,200 \pm 2050$ on the 7th day of culture using cow dung as manure with aeration and supplementary feed.

It is found that the moina harvested in exponential growth phase has more nutritive value. The collected moina can be directly fed to predator larvae or preserved in deep freezer with 10% glycerol for future use (Muthu and Palanichamy 1982).

The resting eggs or dormant cysts of zooplankton are the product of sexual reproduction during unfavorable environmental culture conditions viz., temperature, salinity, oxygen, feed etc. In this study, the population density (above 10,000/l), less feed and the low oxygen level were maintained in moina culture tank to produce the dormant cysts. In this method interestingly more than 50% of fertilized cysts were obtained.

The production rate of resting eggs was almost doubled in culture tanks with the ammonia content of 2-3mg/l which was possible by adding ammonium sulphate. At the same time it was observed that the production of unfertilized cysts was about 80%.

In Semi-Continuous culture method, the yield of resting eggs was higher than the batch culture method.

Muthu and Palanichamy (1982) stated that the cysts stored in glass tube in dry condition were viable up to 6 months. Kandasamy and Palanichamy (1996) found that the cysts stored in saline water of 10-30ppt were viable up to 3 months with less than 10% hatching rate. They also observed that the cell wall of the cysts was eroded

after 3 months of preservation. Chemical treatment with sodium hypochlorite (1ppm) or antibacterial drugs was found to be effective in reducing infection and increasing hatch rates (Balompapurng *et al.*, 1996). Recent research revealed that irradiation of the resting egg with UV light between 320-380 nm most effectively induced the hatching and exposure of the eggs to hydrogen peroxide initiated development of diapausing embryo even in darkness (Hagiwara *et al.*, 1995). The mixed algal feed may increase the cyst production and hatching rate. In our study, the cysts collected and dried without sediments (pure) were not hatched out after a month of preservation. We found that the cysts preserved in dark glass containers at 3-5°C were viable up to 2 years with moderate hatching rate of 20-40% and also viable up to 30 months with 5-10% of hatching. So it is found that this technique is quite suitable for long term preservation of dormant cysts.

Conclusion

Mass production of moina using commercial low cost fertilizers to culture *Chlorella spp* as feed is an economic method for aquaculture systems. This approach could be a better substitute for artemia nauplii in freshwater fish hatcheries and ornamental fish farms to reduce cost of production. Moina harvested during exponential growth phase shows high nutritional value which will fulfill essential nutrients required by the fish larvae. The dormant cyst produced by increasing the population density in moina culture tank at the yield of 50% of fertilized eggs. The produced cyst preserved under dark container viable for more than 2 years for future use.

Acknowledgement

The authors thank to Officer-in-charge, CMFRI, Mandapam Camp, Ramarathapuram district, Tamilnadu for the facilities provided to carry out the research.

References

- APHA : Standard methods for the Examination of water and waste water, 19th edition. American Public Health Association, New York 10 sections (paginated separately) plus figures, D.C., 1040p. (1995).
- Dutta, R. and J. D. Jameson : Production of Moina, *Moina micura* using Cowdung Waste Medium. *Indian J. Ecol.*, 34(1) : 96-97 (2007).
- Jai Sankar Ojha and Gregory A. Howard : Microalgae for aquaculture nutrition. *Fishing Chimes*, 20(9) : 15-22 (2000).
- Kandasamy, D., S. Palanichamy and S. Mohan : Technique for the continuous mass culture of zooplankton. CMFRI, TTC Publication on Live Feed Culture, 1-18 (1997).
- Muthu, M. S. and S. Palanichamy : Continuous culture of moina for larval rearing of marine prawns. *Bull. Cent. Mar. Fish. Res. Inst.*, 43 : 154-155 (1983).
- Padhi, S., P. K. Baliarsingh, B. K. Routray and R. C. Choudhary : The role of freshwater and marine algae in nutrient and maintenance of *Daphnia similis* inculture. *Seaweed Res. Utiln.*, 14(1) : 85-90 (1991).
- Palanichamy, S. : Continuous mass culture of live feed for different stages of prawn and fishes. *Bull. Cent. Mar. Fish. Res. Inst.*, 48 : 117-119 (1996).
- Rottmann, R. W., J. S. Graves, C. Wastson and R. P. E. Yanong : Culture techniques of Moina: The ideal Daphnia for feeding freshwater fish fry. Institute of Food and Agricultural Sciences, University of Florida, Gainesville (2003).
- Santhanam, R., N. Ramanathan and G. Jegathesan : Coastal Aquaculture in India, CBS Publishers, Delhi. pp. 169-175 (1990).
- Thakur, N. K. : Importance of live food organisms in Aquahatcheries. In: N. K. Thakur (ed.) *The Training Manual on Culture of Live Food Organisms for Aquahatcheries*. CAS in Fishery Science. CIFE, ICAR, Mumbai pp. 1-4. (1998).