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Study On The Larval Production Of *Shrimp* Fed On Rotifer Cultured In Different Medium Of *Nannochloropsis* Sp.

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ABSTRACT

The sample was collected from the Manakudy estuary consists of *Nannochloropsis* sp, rotifer (*Brachionus plicatilis*) and shrimp (*Penaeus sp.*) larvae . The grown of *Nannochloropsis* sp. in different culture medium - organic (mixture of liquid cowdung and poultry) and Conway medium, was cultured with rotifer. This experiment was continued till the number of rotifer reached the mid growth phase. The survival of shrimp larvae from Zoea to Mysis stages, when fed the rotifer in different culture medium and maximum was observed in organic medium**.** In this present study the organic culture medium cultured in *Nannochloropsis* sp. have rich oil content this was increase the survival of shrimp larvae.

KEYWORDS

Brachionus plicatilis, Nannochloropsis sp., *Penaeus sp.*, Culture medium

INTRODUCTION

Autotrophic microalgae are one of the main live feed organisms presently in hatcheries having great potential for all growth stages of crustaceans, fish larvae and zooplanktons (Brown *et al*., 1994). Microalgae are rich in protein, vitamins, fat and free amino acids, which play an important role in growth and survival of shell and fin fish larvae (Michael Babu *et al*., 2014). Microalgae cultivation in hatcheries having several drawbacks, the main problem was production cost. The cost of effective algal culture media has been prepared by (Wilkie and Mulbry, 2002) to utilize animal waste as a growth medium.

Organic wastes have rich in organic carbon, nitrogen and phosphorus (Kautto, 2011). Organic carbon is a growth promoting factor for microalgae (Wang *et al*., 2013). Live feed were basic food for culturing of larvae (Richmond, 2004). Shrimp were cultured on phytoplankton and zooplankton as live food in larval stage is essential and important role in improving larval growth and survival (Gallardo *et al*., 1995). Rotifer, *B. plicatilis* are used as live food for marine fish larvae and planktonic crustaceans throughout the world (Watanabe *et al*., 1983). The *Chlorella* cultured in organic culture medium is an effective food to enhance the population density of rotifer than Conway medium cultured *Chlorella* (Gopal *et al*., 2015).

The aim of the present experiment was to study the maximum production of the larva of *Penaeus sp*., when fed on rotifer cultured in organic medium of *Nannochloropsis* sp.

MATERIALS AND METHODS

Collection of *Nannochloropsis* sp, rotifer and shrimp larvae

The desired *Nannochloropsis* sp, rotifer (*Brachionus plicatilis*) and shrimp (*Penaeus sp.*) larvae were collected from the estuary of Manakudy, Kanyakumari district, Tamil Nadu, India.

Selection of organic medium for the production of microalgae

The Cow dung and Poultry manure were allowed to ferment by anaerobic digester. After anaerobic digestion, the supernatants were siphoned out and collect in another two separate buckets and sterilized. The

sterilized supernatant of Cow dung (C) and poultry manure (P) were mixed . Hundred milliliter of estuarine water with 20 ml of organic medium was used as a culture media for the culture of microalgae.

Culture of *Nannochloropsis* sp **in organic medium and Conway medium**

Two groups of sterilized conical flasks were taken and each group contained four conical flasks. One group of conical flasks having 200 ml of Organic Medium and other group of conical flasks having 200 ml of Conway medium were inoculated with 20% of microalgae *Nannochloropsis* sp were added in each separate conical flask. The salinity (15-20ppt) and pH (pH 7-8) were maintained by refractosalinometer and pH meter, respectively. Finally, these setups were placed in front of the white fluorescent tube light at 1000lux. The algal growth was measured in every day using improved Neubauer chamber (Haemocytometer). The algae was grown using organic medium and Conway medium and harvested for feeding to rotifer.

Culture and harvest of rotifer fed with *Nannochloropsis* sp **grown in organic medium and Conway medium**

The harvested algal cells from organic medium and Conway medium were taken in five 500 lit tank (five tank) having 400 or 300 lit of estuarine water and maintained the algal density of 5.23 X 10^5cells/ml. Two liter of rotifer (*B. plicatilis*) culture inoculation having the rotifer count of 10000/ml were added separately in each tank containing the estuarine water. The numbers of rotifers were also noted every day by pipette counting method. This experiment was continued till the number of rotifer reached the mid growth phase and then partially harvested for feeding experiment.

Organic medium and Conway medium grown *Nannochloropsis* sp **feed on rotifer and survival of shrimp larvae from Zoea1 to Mysis1 stages**

Penaeus sp nauplii (stage VI) were stocked in four tanks. Each tank, 20000± 2000 shrimp larvae (nauplii V-VI) was stocked with 50 liter estuarine water. The larvae were fed on rotifer cultured in *Nannochloropsis* sp grown in organic medium and Conway medium in separate tanks for 3 days as long as the larvae revealed to zoea III stage. The zoea 1 (Z1) larvae metamorphosed in to Z2, Z3 and Mysis 1 (M1) after 5, 6 and 7^{th} day respectively. Every day, the percentage of stage conversion was noted and finally, the percentage of survival was calculated on 7^{th} day. The Salinity, light and temperature maintained were 20 ± 5ppt, 5000lux and 30± 2ºC respectively in the culture. Every alternative day, 20% water was exchanged.

Survival of mysis larvae fed on rotifer enriched with *Nannochloropsis* sp **grown in organic medium and Conway medium**

Ten thousand mysis 1 stage shrimp larvae were taken in 100 liter tanks containing 80 liters of estuarine water. The larvae were fed with rotifer cultured from *Nannochloropsis* sp developed from organic medium and Conway medium in separate tanks and reared for 4 days. The larvae mysis1 (M1) metamorphosed into M2, M3 and Post larvae 1 (PL1) from 1^{st} - 5^{th} days after Z3 stage. The percentage of stage conversion was recorded in every day. After 5^{th} day, the percentage of survival was found out.

RESULT AND DISCUSSION

Effect of different LOM for the maximum production density of *Nannochloropsis* sp

Among the nine concentration of LOM, the *Nannochloropsis* sp preferred the LOM5, it gave maximum growth. In *Nannochloropsis* sp, the maximum growth (491.72 x 10^5cells/ml) was obtained in LOM5 and lowest growth (255.71 x 10^5cell/ml) was obtained in LOM1 (Table-1).

Effect of different culture media for the production density of *Nannochloropsis* sp

The algae *Nannochloropsis* sp cultured on Conway medium showed the maximum growth 234.21 X 10^5cells/ml. When as the algae *Nannochloropsis* sp was cultured on organic medium showed the maximum growth of 491.20 X 10^5cells/ml, at mid log phase of the culture. The organic medium grown *Nannochloropsis* sp growth phase duration was longer than the Conway medium grown *Nannochloropsis* sp algae. The organic culture of *Nannochloropsis* sp was higher than Conway medium (Fig 1).

Effect of *Nannochloropsis* sp grown in organic medium and Conway medium for the production density of rotifer

The rotifers growth was mainly affected by quality of the feed. Rotifers fed with *Nannochloropsis* sp grown in Conway medium, showed the maximum density of 254.33rotifers/ml. Rotifers fed with *Nannochloropsis* sp grown in LOM, the maximum density of 466.98 rotifers/ml was recorded (Fig 2).

Effect of organic medium and Conway medium grown *Nannochloropsis* sp on stage conversion and percentage of survival for shrimp larvae from Zoea1 to Mysis1 stages

Shrimp larvae of zoea 1 (Z1) was fed with Conway and Organic Medium (OM) grown *Nannochloropsis* sp . After one day, the Z1 larvae metamorphosed into Z2, the percentage of conversion were 84.00 and 93.50% respectively. In second day, the Z2 metamorphosed into Z3, the percentage of conversion were 86.31 and 94.08% respectively. After 3rd day, the Z2 metamorphosed into mysis 1 (M1), the percentage of conversion were 82.75 and 94.31% respectively. After metamorphosis of Z1 to M1 the percentage of survival in Conway medium and LOM grown microalgae *Nannochloropsis* sp was 60.00 and 83.00 respectively(Table-2).

Nutritive efficiency of Rotifer enriched with organic medium and Conway medium grown *Nannochloropsis* sp on metamorphosis and survival of shrimp larvae (M1-PL1)

The mysis larvae (M1) of shrimp stage were fed with rotifer enriched with *Nannochloropsis* sp grown in Conway medium and LOM. After one day, the M1 metamorphosed into M2, when the percentages of conversion were 79.00 and 85.40% respectively. In second day, the M2 metamorphosed into M3, the percentages of conversion were 74.30 and 89.58% respectively. After 3rd day, the M2 metamorphosed into post larvae1 (PL1), the percentages of conversion were 67.97 and 76.60% respectively. After metamorphosis of M1 to PL1 the total percentages of survival in rotifer enriched with *Nannochloropsis* sp grown in Conway medium and organic medium were 39.90 and 58.60 respectively(Table-3).

Table 1. Growth of *Nannochloropsis* sp in Cow dung and Poultry manure

Days	Growth of Microalgae X 10^5 cells/ml								
1	021.64±0.81	022.64±1.24	022.76±1.18	021.12±1.74	021.78±0.97	021.78±1.24	021.45±1.24	022.22±1.67	021.34±1.18
2	048.72±0.84	046.37±0.97	054.34±1.84	052.71±1.62	058.54±1.16	054.76±1.26	056.73±2.14	047.61±1.62	041.97±1.18
3	087.63±1.42	094.35±1.04	098.64±2.49	142.36±2.86	136.75±3.62	118.72±2.27	127.47±1.47	098.24±1.97	094.37±1.38
4	149.42±2.35	173.14±2.62	182.46±3.73	247.66±4.08	267.20±4.84	205.18±3.86	249.62±3.78	217.24±2.18	196.32±2.42
5	194.39±3.56	283.48±4.24	341.28±1.97	379.44±4.74	386.35±5.63	346.34±4.68	373.64±4.47	304.34±3.46	261.38±4.78
6	241.44±2.15	304.76±4.18	371.59±4.63	468.74±5.74	463.22±4.73	398.79±2.97	432.71±3.84	312.73±4.94	328.47±3.18
7	255.71±3.86	301.38±2.69	328.43±5.42	468.29±4.57	491.72±6.76	403.62±4.18	424.18±5.24	282.79±2.78	329.58±4.39
8	268.24±4.62	258.18±3.74	267.87±3.96	396.71±4.79	412.34±7.34	364.32±3.76	390.92±3.76	302.52±3.59	283.24±3.84

Table 2. Effect of organic medium and Conway medium grown Nannochloropsis sp. fed rotifer on larval survival of shrimp in zoea l stages

culture medium	Shrimp zoea larvae			% of total survival
	% of stage conversion			
	Z1-Z2	Z2-Z3	Z3-M1	Z1-M1
Conway	84.00±8.23	86.31±9.08	82.75±7.47	60.00±4.22
Organic medium	93.50±9.34	94.12±7.48	94.31±9.24	83.00±8.24

Table 3. Effect of organic medium and Conway medium grown Nannochloropsis sp. fed rotifer on survival of shrimp Mysis larvae

Culture medium	shrimp Mysis larvae			
	% of stage conversion			% of total survival
	M1-M2	M2-M3	M3-PL1	M1-Pl1
Conway	79.00±8.72	74.30±6.62	67.97±6.87	39.90±3.74
Organic medium	85.40±7.94	89.58±8.65	76.60±7.96	58.60±5.78

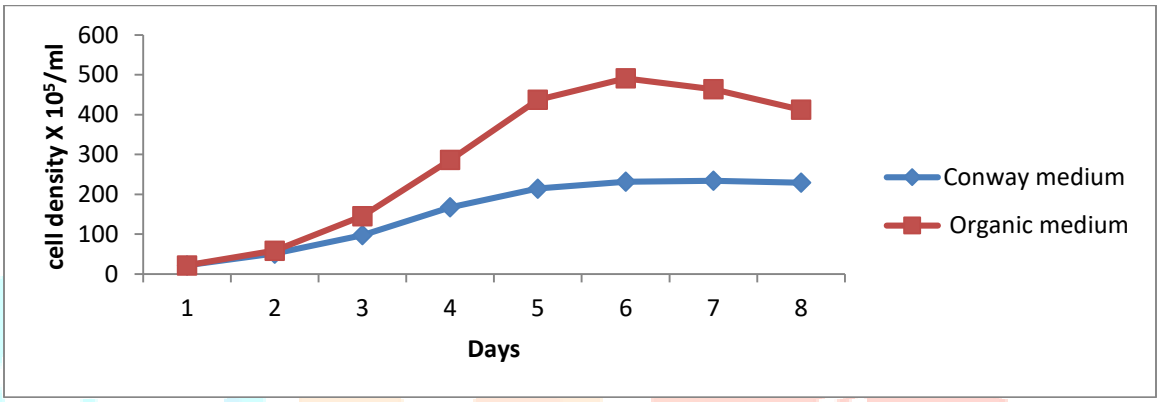

Fig 1. Effect of different culture media for the production density of *Nannochloropsis* sp.

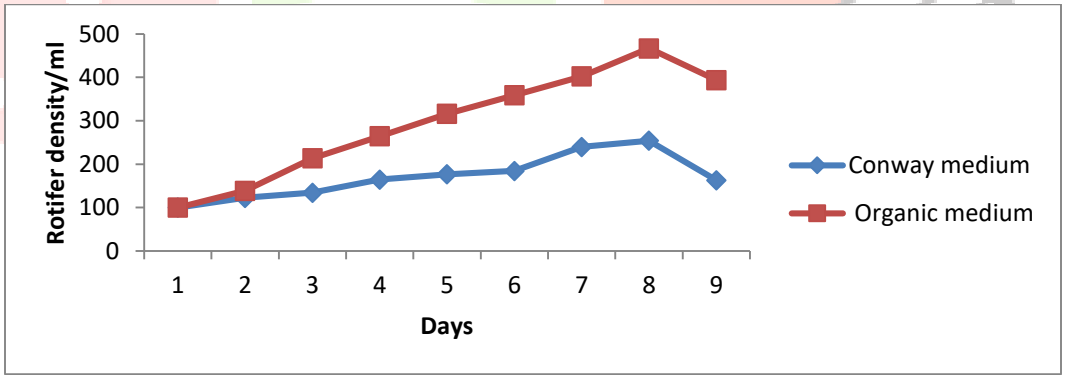

Fig 2. Effect of *Nannochloropsis* sp grown in different culture media for the population density of rotifer

In aquaculture, the microalgae directly or indirectly were used as an excellent first food for early stages of farmed shrimp (Rosenberry, 1998). The usage of live feed such as phytoplankton and zooplankton was a key aspect for the success of fish and crustacean larvae culture in nursery; however, the use of the food source has high economical costs (Lin *et al*., 2009). The expensive of larval rearing was reduced by only one way that was low cost food preparation for larvae. The lot of researcher's says that the Organic waste was chief and best for cultured microalgae. The low cost organic waste containing rich micronutrients such as nitrogen and phosphorus were used as essential nutrients for microalgal growth (Iyoyo *et al*., 2010).

The algae were rapidly grown in OM than the Conway medium. Since 2013, Shweta and Samuel, the organic Cow Dung Ash Medium (CDAM) was used as growth medium for algae *Spirulina platensis* in pure form without any supplemented nutrients and the algae growth was higher than the prescribed CFTRI medium.

In this experiment the OM was purified and optimized by the growth of microalgae. The microalgae *Nannochloropsis* sp was cultured on organic medium and Conway medium and the maximum was grown in organic medium.

Microalgae manufacture more complex nutritive molecules including proteins, starches, fatty acids and oils in reasonable quantities hence is an important part of crustacean and fish larval nutrition (Brown *et al*., 1997; Stahl, 2009). In larval rearing quantity of oil was playing an important role in survival and growth of shrimp larvae.

The organic medium cultured microalgae also contain rich oil content. The oil content was transmit and to stimulate the growth and survival of zooplankton (rotifer) and shrimp larvae (Z1-M1). This present study result was the organic medium grown algae fed rotifer and shrimp larvae growth and survival higher than that of Conway medium grown algae fed rotifer and shrimp larvae.

The usage of rotifers as live feed has demonstrated to increase aquaculture yields, in particular *Brachionus* sp. (Campaña-Torres *et al*., 2008). Algae *N. oculata* was important feed used for *Brachionus* sp. culture (Spolaore *et al*., 2006). The fish larvae were fed with microalgae and rotifer, because artificial feeds were not taken up by shrimp larvae, the uptake of therapeutics and nutrients through the algal and rotifer feed. In this present study the LOM medium cultured algae have rich oil content this was increase the survival of shrimp larvae.

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