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Selection of suitable media and intervals of media inoculation for culturing Tubificid worms

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Abstract

Tubificid worms are aquatic invertebrates, belonging to the class Oligochaeta and family Tubificidae, used as an important live food for fishes. The study was conducted to culture Tubificid worms under running water in order to develop a suitable culture media and an optimum duration of media inoculation for culturing Tubificid worms. The worms were cultured under two experiments in cemented culvert system (160×25×10 cm^3) for 90 days. In the first experiment the worms were cultured in three different media designated as treatment-I, treatment-II and treatment-III. The highest yield (503.39±22.98 mg cm^{-2}) was found at 70^{th} day of culture duration in the culture media containing a mixture of 35% mustard oil cake, 20% wheat bran, 25% cow-dung and 20% fine sand (treatment-III). Only 1.99 kg media ingredients valued BDT 29.85 were needed to yield 1 kg worms. In the second experiment, the worms were cultured at three different intervals of media inoculation i.e., 6, 10 and 15 days interval designated as treatment-I, treatment-II and treatment-III respectively using the media found best in the first experiment. Inoculation of media at 10 days interval showed significantly (P<0.01) higher production (488.94±5.60 mg cm^{-2}).

Keywords: Tubificid worms, Oligochaeta, Culture media, Intervals of media inoculation

Introduction

Tubificids, aquatic oligochaete, are reddish in colour found mostly in old canals and drains in towns where steady and continuous water flow exists and where large amounts of organic detritus are present. They are very small usually having a length of 3-4 cm (Mellanby, 1953; Jordan and Verma, 1978). They make a kind of tube for themselves out of mucus and mud particles, which then sink into the ground. The anterior part of the body settles in the tube while the rest of the body waves above the ground in a breathing movement. Majority of the aquatic species are common in the mud and debris of pools, ponds, streams, lakes and sewage canals where considerable organic matter is undergoing decay. Tubificids occur even in the depth exceeding one meter, and in the deep water of lakes there are sometimes more than 8,000 individuals m^{-2} (Barnes, 1966).

These worms can be one of the best quality live foods for fish because of their high food value (5575 cal g^{-1} dry weight; Giere and Pfannkuche, 1982). Phillips and Buhler (1979) documented significantly higher growth rates of rainbow trout (*Salmo gairdneri)* fed on *Tubifex* sp. over standard pellets. Buddington and Doroshov (1984) also showed that white sturgeon (*Acipenser transmontanus)* grew 40% larger when fed *Tubifex* sp. compared to inanimate pellets. Alam and Mollah (1988) reported significantly higher survival rate and 10 times more growth of catfish (*Clarias batrachus* L) larvae fed Tubificid worms over those fed formulated dry feed. The importance of Tubificid worms as a source of natural food for the fish larvae was also demonstrated for species like *Clarias macrocephalus* (Mollah and Tan, 1982), *Clarias gariepinus* (Mollah, 1991) and *Channa striatus* (Mollah *et al.*, 2009). Tubificid worms have also been proved as one of the best and the cheapest live foods for various types of laboratory animals including planaria, leeches, dragonfly, aquatic beetle larvae, prawns and frogs. Ecologically, it is an important source of food for leeches, crustaceans, insects and fishes. Supplementation of Tubificid worms with factory feed increases the appetite of fish and they are considered as rare delicacy. Moreover, use of Tubificid worms as aquarium and/or ornamental fish food has been a common phenomenon in many countries including USA and USSR since long ago (Lietz, 1987). To the aquarium fish keepers the demand of these worms is considerably high. Some hatcheries also use these worms to certain extent for larvae nursing purposes. Unfortunately no suitable feed has been developed for the larvae to replace the live food to date so far as the growth and survival rate are concerned. Therefore finding a suitable culture media with optimum interval of media inoculation for culturing these worms was felt important.

Materials and Methods

Two experiments were conducted in cemented culvert (160×25×10 cm^3) system under a tin-shed to develop a suitable culture media and to assess the suitable interval of media inoculation for the production of Tubificid worms.

Experiment-I: Selection of suitable culture media

The worms were cultured in three different media (Table 1) to conduct a 3×5 factorial design (3 treatments each with 5 replications) for 90 days.

Experiment-II: Determination of suitable time interval of media inoculation to the culture system

This experiment was designed based on experiment-I and was carried out for a period of 90 days. Here the worms were cultured in three different intervals of media inoculation i.e., 6, 10 and 15 days interval under three treatments namely treatment-I, treatment-II and treatment-III.

Culture procedure

Before starting the experiment, the culture culverts were washed and cleaned thoroughly with fresh water. Water was continually supplied from the deep well to a water reservoir tank with which the culverts were connected a by stop cork. Each culvert was facilitated with inlet (porous PVC pipe of 180 cm long and 1 cm^2 diameter) and outlet to assist in the renewal and removal of water concurrently. The required amount of locally available ingredients mentioned in Table 1 were collected and measured by a laboratory balance (TANITA, KD-160) on a proportional basis to make up 1000 g of media for each culvert. The ingredients were then mixed with sufficient amount of water in three different fibreglass tanks and kept for seven days for decomposition. Subsequent mixing was done every morning and evening for better mineralization. Then required amount (250 mg cm^{-2}) of the well-mixed media was suffused to each of the culvert using a small plastic bowl.

Table 1. Combination of different media ingredients in three treatments

Media ingredients	% of ingredients		
	Treatment-I	Treatment-II	Treatment-III
Mustard oil cake	20	25	35
Wheat bran	35	30	20
Cow-dung	25	25	25
Sand	20	20	20

Live Tubificid worms were collected from the drains of Bangladesh Agricultural University (BAU) campus, Mymensingh, Bangladesh. Collected worms were cleaned using flowing water and conditioned over 24 h in a flow-through system. At first, media were introduced to the culverts and water flow was adjusted at a rate of 1.24±0.33 L min^{-1} to keep the dissolved oxygen above 5 ppm. After 24 h the collected worms were inoculated at the rate of 1.25 mg.cm^{-2} (i.e., 5 g $culvert^{-1}$). The periodic supply of culture media was done once in every 10 days at 1000 h at the rate of 250 mg cm^{-2} in respective culverts. Water flow was stopped during media addition. Following water quality parameters were recorded during the experimental period:

a. **Water temperature:** Water temperature of the culture culverts was recorded with digital thermometer at 1000 h once in every 10 days before sampling.
b. **Dissolved oxygen:** Dissolved oxygen (DO) was determined with the help of Dissolved Oxygen Meter (Model: DO- 5509) once in every 10 days before sampling.
c. **pH:** Water pH was measured by using portable digital pH Meter (Model: HI 98127) once in every 10 days before sampling.

Collection of worms from each culvert was initiated after 40th day of the inoculation of Tubificid worms at every 10 days interval to determine the growth and multiplication as well as the production of the worms (Fig. 1) before the introduction of new media. Tubificid worms were collected by a glass tube having a diameter of 2.2 cm from three randomly selected places of each culvert. They were cleared from their respective media by water flow and finally parted from the unwanted particles using forceps and dropper. Separated worms were dried with tissue paper and weighed using a Matler electric balance graduated in 0.000 g. Data were analyzed through one-way analysis of variance (ANOVA) followed by Turkey's HSD post hoc for multiple comparisons. Statistical analysis was performed using the statistical software SPSS version 11.5 with the level of significance at $P<0.05$.

Results and Discussion

Experiment-I: Selection of suitable culture media

The standing biomass of Tubificid worms in three different culture media is presented in Table 2. The highest standing biomass of 503.39±22.98 mg cm^{-2} was found in treatment-III on the 70th experimental day where the quantity of the mustard oil cake was the highest (35%) compared to the other treatments. The standing biomass of treatment-I was significantly ($P<0.01$) different from those of treatment-II and treatment-III throughout the experiment except on the 70th experimental day (Table 2). In treatment-II and treatment-III a gradual increase in standing biomass of Tubificid worms was found up to the 70th experimental day followed by a decrease in biomass up to the end of the experimental periods (90 days). Statistical analysis by ANOVA showed that there was no significant difference ($P<0.01$) in production between treatment-II and treatment-III, though the standing biomass of Tubificid worms in treatment-III was higher than that of treatment-II from the starting to the end of the experiment. However, treatment-I showed the same trend of increase in biomass up to the 80th day showing a peak of 422.06±1.33 mg cm^{-2} on the 80th experimental day followed by an abrupt decline in the biomass of 313.22±5.03 mg cm^{-2} at the 90th day. The result showed that the standing biomass of treatment-I faced troublesome situation of lower population throughout the experimental period due to the effect of media, where the quantity of mustard oil cake was the lowest (20%) compared to the other media.

Table 2. Standing biomass (mg cm^{-2}) of Tubificid worms in three different treatments during 90 days experimental period (mean±SD)

Treatments	Experimental period in days					
	40	50	60	70	80	90
I	115.65 ±10.39^{c}	207.92 ±23.34^{b}	295.16 ±4.04^{b}	344.83 ±11.11^{b}	422.06 ±1.33^{b}	313.22 ±5.03^{a}
II	204.46 ±30.50^{b}	300.56 ±26.92^{a}	370.44 ±22.47^{a}	450.01 ±20.09^{a}	376.90 ±12.24^{a}	265.40 ±3.46^{b}
III	280.26 ±20.03^{a}	352.45 ±23.52^{a}	426.26 ±18.97^{a}	503.39 ±22.98^{a}	404.13 ±1.48^{ab}	319.19 ±6.51^{a}

* Values with different Superscripts in a vertical column are significantly different (one way ANOVA followed by Tukey test, $P<0.01$).

In this study the choice of media substrates containing a mixture of various ratio of mustard oil cake, wheat bran, cow-dung and fine sand for culture of Tubificid worms and the rate of media application was made on the basis of previous studies conducted by Marian and Pandian (1984) and Ahamed and Mollah (1992). A comparison of the yield of these worms indicated that the media containing 35% mustard oil cake, 20% wheat bran, 25% cow-dung and 20% sand (treatment-III) was the best as it produced 503.39±22.98 mg cm^{-2} of Tubificid worms on 70th day (Table 2). Ahamed and Mollah (1992) reported that the media containing 20% mustard oil cake, 35% wheat bran, 25% cow-dung and 20% sand gave better production (419.4 mg cm^{-2}) of Tubificid worms compared to a substrate containing 75% cow-dung and 25% fine sand as indicated by Marian and Pandian (1984). However, Marian and Pandian (1984) also reported a production of 200 mg cm^{-2} worms.

Mustard oil cake, wheat bran, soybean meal, cow-dung and sand are important media ingredients for culturing Tubificid worms. Ahamed and Mollah (1992) observed better growth when mustard oil cake and wheat bran were used as media ingredient while present study demonstrated that mustard oil cake has a definite effect on the culture of these worms, probably because of containing various nutrients like minerals and organic matter (Ahamed and Mollah, 1992). The production in treatment-I was lower due to the lower quantity of mustard oil cake in comparison to other treatments.

Fecundity of *Tubifex tubifex* depends on the temperature, rate of water flow and organic content of the culture media (Marian and Pandian, 1984). Sexual maturity is more rapid at a higher temperature and enough dissolved oxygen content when adequate organic carbon (especially mustard oil cake) is present in the culture media. In the present study water temperature, dissolved oxygen and pH of the culverts were suitable and in productive range as found by Davis (1982), Poddubnaya (1980) and Li RenXi (2001) and ranged from 24.3 to 26.5°C, 7.0 to 7.4 ppm and 7.1 to 7.4 respectively.

Ahamed and Mollah (1992) stated that 2.85 kg raw materials were required for 1 kg worm production against 18 kg and 25 kg cow-dung reported by Marian and Pandian (1984) and Marian *et al.* (1989) respectively. The present study required only 1.99 kg raw materials (35% mustard oil cake, 20% wheat bran, 25% cow-dung and 20% fine sand) valued BDT 29.85 for 1 kg worms which proved the superiority of this media for the production of Tubificid worms compared to the previous ones.

Experiment-II: Determination of a suitable time interval of media inoculation to the culture system

The standing biomass of Tubificid worms at three different intervals of media inoculation into the culverts are presented in Fig. 2. The production of the worms at three different treatments showed significant difference ($P<0.01$) throughout the experimental period (Fig. 2). Results analyzed by ANOVA with multiple comparisons revealed that the intervals of media application into the culverts have significant effect on the growth and production of Tubificid worms. The standing biomass of treatment-II was significantly ($P<0.01$) higher from that of treatment-III showing a peak production of 488.94±5.60 mg cm^{-2} on the 70^{th} experimental day (Fig. 2). In treatment-II, a gradual increase in standing biomass was spectacular up to the 70^{th} experimental day before showing a decline in biomass up to the end of the experimental periods (90 days). In treatment-I, the standing biomass of Tubificid worms reached peak point earlier (60^{th} experimental day) compared to other treatments. Recurrent application of media to the culverts might be responsible for this. After 60^{th} day abrupt falling trend of standing biomass in treatment-I indicated that higher decomposition with low dissolved oxygen might be responsible for this. The standing biomass of the worms in treatment-III reached its pinnacle later (80^{th} experimental day) compared to other treatments. This delay occurred due to 15 days interval of media application, which led to run out nutrients rapidly. Thus, the growth of Tubificid worms hampered.

The experiment for determining the suitable interval of media application to the culture system was based on the previous study conducted by Mollah and Hossain (1995). They found the highest production of 556.25±21.87 mg cm^{-2} when the media was supplied at 6 days interval compared to those supplied at 10 days and 15 days interval. In the present study, media introduction to the culverts between 6 days and 10 days interval showed little incongruity in terms of production. The production in treatment-I and treatment-II were higher than that of treatment-III. The culverts in treatment-I and treatment-II were rich in organic matters compared to that of treatment-III. According to Huet (1979), the colonies of *Tubifex tubifex* reproduced in the mud rich organic matter. In the present study, although the availability of organic matter in treatment-I was the highest among those of treatment-II and treatment-III, the production in treatment-I (371.40±18.04 mg cm^{-2}) was lower than that of treatment-II (488.94±5.60 mg cm^{-2}) and treatment-III (335.37±17.19 mg cm^{-2}). The low production in treatment-I may be due to the scarcity of dissolved oxygen created by higher decomposition of organic matter. On the other hand, treatment-III showed the least production indicating the absence of required amount of organic matter due to longer gap of media application. So, it is inferred that the media inoculation at 10 days interval is better than that of 6 days interval in terms of production. Treatment-II is also more suitable in terms of economic consideration as it reduced the media cost compared to treatment-I maintaining the comparable production.

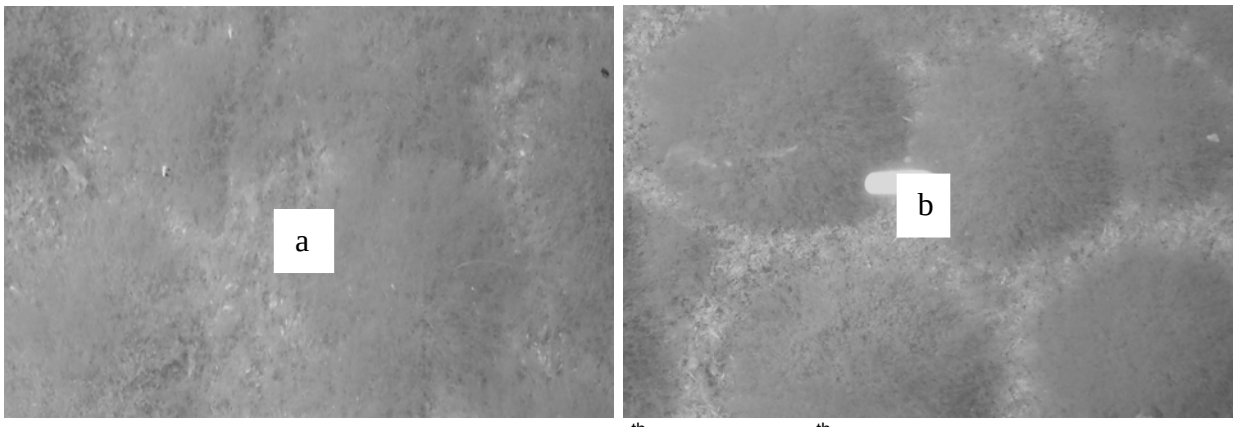

Fig. 1. Colony of Tubificid worms at a) 50th day and b) 70th day of culture period.

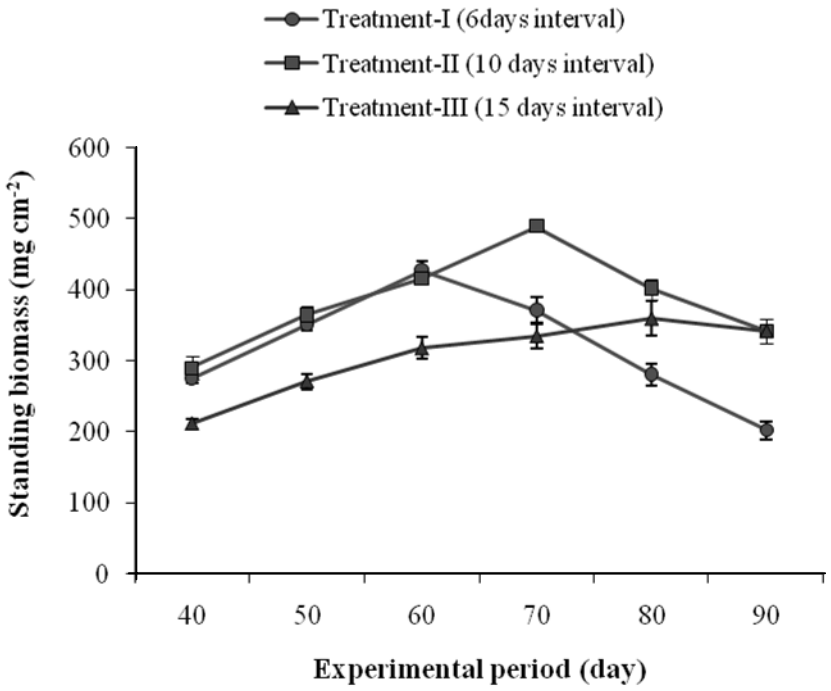

Fig. 2. Standing biomass (mg cm^{-2}) of Tubificid worms at three different intervals (6, 10 and 15 days) of media inoculation.

During the experiment temperature, dissolved oxygen and pH of water of the culverts were suitable ranging from 24.3 to 26.8°C, 7.1 to 7.4 ppm and 7.1 to 7.3 respectively.

Conclusion

Although the production of Tubificid worms is increased with congenial water temperature, dissolved oxygen, pH and water flow rate, suitable media and appropriate interval of media application to the culture system have substantial impact on the production of the worms.

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