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Development of a Suitable Culture Medium for the Production of Tubificid Worms

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Abstract

Tubificid worms (Tubificidae) are one of the best quality live foods in intensive aquaculture widely used for feeding certain fish larvae to produce stockable sized seeds in the hatcheries as well as in the rearing of aquarium/ornamental fishes. This study was undertaken to find suitable culture media for sustainable production fulfilling the increasing demand of tubificid worms. The worms were cultured in cement culvert system (160×25×10 cm^3) for 90 days using four different media designated as treatment-I, treatment-II, treatment-III and treatment-IV. Continuous water flow at the rate of 1.24±0.32 $L \cdot min^{-1}$ was maintained. The highest yield (P<0.05) of 999.16±40.29 $mg \cdot cm^{-2}$ was obtained at 70th day culture duration in the culture media containing a mixture of 20% mustard oil cake, 20% wheat bran, 30% soybean meal, 20% cow-dung and 10% sand soaked with rice gruel (treatment-III). Only 1.01 kg media were needed to yield 1 kg worms indicating the suitability of this medium for large scale production.

Introduction

Tubificid worms, also called the sludge worms, or sewage worms are aquatic invertebrates, belonging to the class Oligochaeta and family Tubificidae. They reside in the sediments of lakes, rivers, and in flowing waters of industrial sewerage, canals, and drain. They are reddish in colour and have the same fundamental structure of the common terrestrial earthworms. They are small and usually 3-4 cm long (Mellanby, 1953; Jordan and Verma, 1978). They are the most widely distributed and abundant group of Annelids in freshwater (Brinkhurst and Kennedy, 1965; Birtwell and Arthur, 1980). They are found to grow vigorously, forming reddish colonies in the mud, particularly when it is rich in organic detritus (excretory products of human beings and other animals, rotten leaves, rotten food particles). These worms ingest sediments and gain nutrition by selectively digesting bacteria therein and absorbing molecules through the body wall (Rodriguez et al. 2001).

Tubificid worms are considered as one of the nutritious foods for fish (Marian, 1982) as they contain high food value (5,575 $cal \cdot g^{-1}$ dry weight; Giere and Pfannkuche, 1982). Percentage of crude protein, crude lipid and ash content of tubificids are 63.32, 28.84 and 7.95 respectively (Mollah and Ahamed, 1989). Tubificid worms are used as a good source of protein and the amino acid profile of the proteins in tubificid worms are very suitable for fish (Jhingran, 1982). The superiority of tubificid worms to any formulated feed in fish larvae and fry production in terms of

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growth and survival have been demonstrated in many fish species (Phillips and Buhler, 1979; Mollah and Tan, 1982; Buddington and Doroshov, 1984; Alam and Mollah, 1988; Mollah, 1991; Mollah et al. 2009).

Marian and Pandian (1984) attempted to study the culture and harvesting techniques for *Tubifex tubifex* on different substrates but refinement seemed mandatory to make the technique suitable as well as economical for mass production. The aspects that needed immediate attention are whether a better medium could be obtained than the ones identified by Ahamed and Mollah (1992) and Mosharaf (2009). Both these authors work in the same laboratory conducting their experiments under similar conditions. Ahamed and Mollah (1992), a pioneer researcher in Bangladesh advocated a medium comprising 20% mustard oil cake, 35% wheat bran, 25% cow-dung and 20% sand as the best for tubificid worm production. However, recently Mosharaf (2009) used media comprising 35% mustard oil cake, 20% wheat bran, 25% cow-dung and 20% sand and reported higher production (503.39 $mg \cdot cm^{-2}$) compared to Ahamed and Mollah (1992). In the present study, together with the above two media, a different approach using soybean meal (30%) and rice gruel, was made to ascertain their effect on the production of tubificid worms. Therefore, a medium comprising 20% mustard oil cake, 20% wheat bran, 30% soybean meal, 20% cow-dung and 10% sand soaked with rice gruel and another medium having the same ingredients soaked with water instead of rice gruel was used.

Materials and Methods

The experiment was conducted with a view to developing a suitable medium for sustainable production of tubificid worms. The worms were cultured for 90 days in cement culvert (160×25×10 cm^3) system under a tin shed to protect the worms from rain and sunlight or any other natural disturbance. Sixteen culverts were used to conduct a 4×4 factorial design (4 treatments each with 4 replications) where media were the only experimental variable (Table 1).

Table 1. Combination of different media ingredients in four treatments.

Media Ingredients	Ingredients (%)			
	Treatment-I	Treatment-II	Treatment-III	Treatment-IV
Mustard oil cake	20	35	20	20
Wheat bran	35	20	20	20
Soybean meal	-	-	30	30
Cow-dung	25	25	20	20
Sand	20	20	10	10
Water	As required	As required	-	As required
Rice gruel	-	-	As required	-

To wet the media ingredients, sufficient amount of water was used for treatment-I, treatment-II and Treatment-IV, and rice gruel for treatment-III in four separate fibreglass tanks.

Preparation of culture unit

Before starting the experiment, the culture culverts were washed and cleaned thoroughly with fresh water. Culverts were connected with a water reservoir tank by stop cork where the water was constantly supplied from the deep well. In order to facilitate renewal and removal of water concomitantly, an inlet and an outlet were provided with each culvert. A porous PVC pipe (180 cm long and 1 cm^2 diameter) set up longitudinally over each culvert with the help of bamboo sticks acted as inlet for ensuring the required water flow rate and dissolved oxygen concentration (Fig. 1).

Collection of ingredients for media preparation

Locally available ingredients were used for media preparation. Mustard oil cake, wheat bran and soybean meal were purchased from Mymensingh, Bangladesh. Cow-dung (about 7 days old) was bought from the Dairy Farm of Bangladesh Agricultural University (BAU) and sand was collected from the Old Brahmaputra River flowing by the eastern side of BAU campus. Rice gruel was collected from the kitchen of the student's residential halls of BAU, Mymensingh, Bangladesh.

Fig. 1. The culvert system.

Analysis of proximate composition of media ingredients

Proximate composition of rice gruel was determined following the standard methods given by the Association of Official Analytical Chemists (AOAC, 1980). The proximate composition of the different ingredients is shown in Table 2.

Media preparation

The required amount of ingredients were measured by a laboratory balance (TANITA, KD-160) on a proportional basis to make up 1,000 g of media for each culvert and mixed thoroughly with a bamboo stick with sufficient amount of water and rice gruel in separate fibreglass tank as mentioned in Table 1. The mixture was kept in this form for 7 days (Hossain et al. 2011) for decomposition before introducing into the culture unit. Subsequent mixing was done twice a day for better mineralisation. After 7 days, 250 $mg \cdot cm^{-2}$ of the well-mixed media were distributed to each of the culverts with the help of a small plastic bowl.

Table 2. Proximate composition of media ingredients (% dry matter basis).

Ingredients	Dry matter (%)	Protein (%)	Lipid (%)	Ash (%)	Crude fibre (%)	Nitrogen free extract (NFE)	References
Soybean meal	90.14	45.29	2.93	9.74	-	42.03	Sarowar and Mollah, 2009
Mustard oil cake	91.28	30.13	6.99	11.58	-	51.44	
Wheat bran	89.83	14.19	3.87	4.89	-	77.05	
Rice gruel	1.68	6.69	4.37	3.08	1.15	84.71	The present study

Collection of experimental tubificid worms

Wild tubificid worms were collected from the residential area of BAU campus. The collected worms were cleaned by using continuous flow of water and held in a flow-through system for conditioning over 24 hr before inoculation into the culverts for culture.

Inoculation of tubificid worms

The inoculation of the worms was done 24 hr after the media introduction. The collected worms were inoculated at the rate of 1.25 $mg \cdot cm^{-2}$ (i.e., 5 $g \cdot culvert^{-1}$) (Ahamed and Mollah, 1992). They were spread over the media homogeneously as much as possible in each of the culvert.

Maintenance of water flow

Continuous water flow at the rate of 1.24±0.32 $L \cdot min^{-1}$ was maintained to keep the dissolved oxygen above 5 ppm. The water flow rate was maintained by the adjustment of the stop cork of the PVC pipes. Water depth over the media was maintained at 4 cm by a depth regulator.

Periodic supply of media

The periodic supply of culture media was started from the 10^{th} day of worm inoculation (Ahamed and Mollah, 1992). The prepared media were introduced at the rate of 250 $mg \cdot cm^{-2}$ to the respective culverts once in every 10 days at 1000 hr. Total quantity of media was spread homogenously throughout the culverts. Water flow was stopped during media addition.

Procedure of sampling, clearing and weighing of tubificid worms

After around 40 days of the inoculation of the tubificid worms into the culture system, the worms grew and formed colonies. To determine the growth and multiplication as well as the production, sampling was done at 10-day intervals starting from 40^{th} day of worms inoculation. Tubificid worms were collected by a sampler (glass tube) having a diameter of 2.2 cm with water and media from three randomly selected places of each culvert before the introduction of new media. The worms were cleared from their respective media by water flow. Final separations of the unwanted particles were done by using forceps and dropper. Separated tubificid worms were dried with tissue paper and their weight were taken by Mettler electric balance (METTLER TOLEDO, PG503-SDR, Switzerland).

Water quality parameters

Water temperature of the culture culverts was recorded with a digital thermometer at 1000 hr once in every 10 days before sampling. Dissolved oxygen (DO) and pH of water were determined with the help of a dissolved oxygen meter (Model: DO 5509, Lutron) and a portable digital pH meter (Model: HI 98127, HANNA) respectively once in every 10 days before sampling.

Statistical analysis

Data were analysed by using one-way analysis of variance (ANOVA). Significant results were further tested using Tukey's HSD post hoc to identify significant difference between means. Data have been presented as mean±SD and the statistical analysis was performed using the statistical software SPSS version 11.5 with the level of significance at $P<0.05$.

Results

The standing biomass of tubificid worms during the 90 days experimental period in four different treatments is presented in Table 3.The average standing biomass of tubificid worms in four treatments were 502.52±20.68 $mg \cdot cm^{-2}$, 506.49±19.67 $mg \cdot cm^{-2}$, 999.16±40.29 $mg \cdot cm^{-2}$ and 674.13±42.18 $mg \cdot cm^{-2}$ in treatment-I, treatment-II, treatment-III and treatment-IV respectively at 70^{th} experimental day. ANOVA test results indicated that there was significant difference in mean total calculated productions among the four different treatments (Table 3). Statistical analysis showed that the standing biomass of tubificid worms was significantly higher ($P<0.05$) in treatment-III than those of treatment-I, treatment-II and treatment-IV throughout the experiment (Fig. 2). There was no significant difference ($P<0.05$) between treatment-I and treatment-II (Fig. 2).

Table 3. Standing biomass ($mg \cdot cm^{-2}$) of tubificid worms in four different treatments during 90 days experimental period (mean±SD).

Treatments	Experimental period in days					
	40	50	60	70	80	90
I	146.97 ±3.03^{c}	310.37 ±19.81^{c}	429.99 ±28.59^{c}	502.52 ±20.68^{c}	460.30 ±26.50^{c}	384.07 ±25.11^{c}
II	226.28 ±32.33^{b}	340.83 ±26.44^{c}	448.37 ±28.52^{c}	506.49 ±19.67^{c}	461.25 ±30.65^{c}	394.25 ±41.68^{c}
III	360.63 ±13.89^{a}	616.65 ±16.58^{a}	759.71 ±29.89^{a}	999.16 ±40.29^{a}	889.09 ±68.53^{a}	825.18 ±59.28^{a}
IV	255.29 ±22.42^{b}	417.42 ±32.34^{b}	559.07 ±38.01^{b}	674.13 ±42.18^{b}	585.19 ±54.84^{b}	501.44 ±30.72^{b}

Values with different superscripts in a vertical column are significantly different (one way ANOVA followed by Tukey test, $P<0.05$).

The peak production of tubificid worms was found at 70th day sampling in all the media treatments (Fig. 2). In all cases, a gradual increase in the 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 period (90th day).

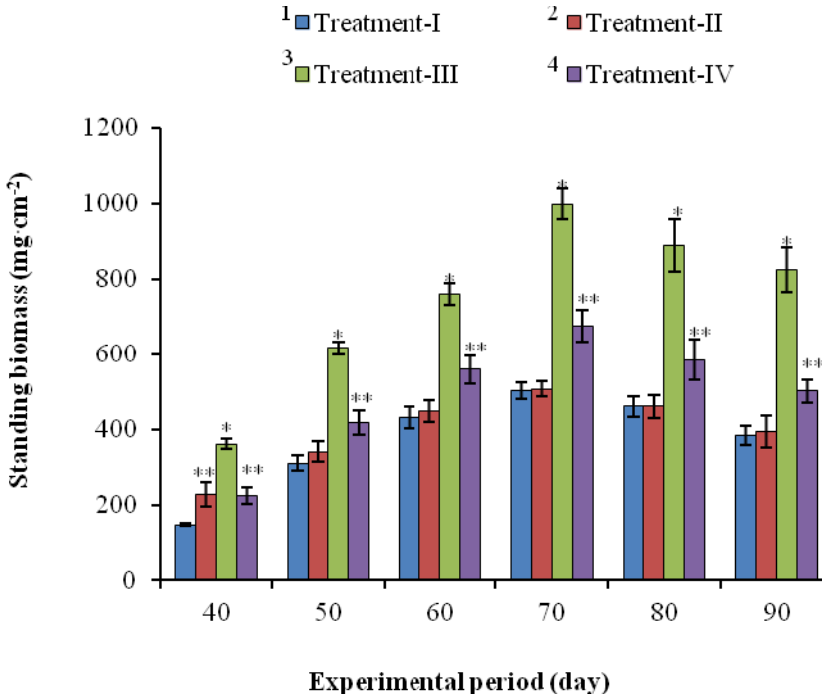

Fig. 2. Standing biomass (mg·cm^{-2}) of tubificid worms in four different treatments during 90 days experimental period (mean±SD). *P<0.01and **P<0.05.
1: 20% mustard oil cake, 35% wheat bran, 25% cow-dung and 20% fine sand
2: 35% mustard oil cake, 20% wheat bran, 25% cow-dung and 20% fine sand
3: 20% mustard oil cake, 20% wheat bran, 30% soybean meal, 20% cow- dung and 10% fine sand wet with rice gruel
4: 20% mustard oil cake, 20% wheat bran, 30% soybean meal, 20% cow- dung and 10% fine sand wet with water

To make the culture sustainable, from the 40th experimental day tubificid worms were harvested at the rate of 40 mg·cm^{-2} from all the treatments (Ahmed et al. 1998). Treatment-III showed significantly higher standing biomass (825.18±59.28 mg·cm^{-2}) compared with treatment-I (384.07±25.11 mg·cm^{-2}), treatment-II (394.25±41.68 mg·cm^{-2}) and Treatment-IV (501.44±30.72 mg·cm^{-2}) (Table 4).

Table 4. Total calculated production ($mg \cdot cm^{-2}$) of tubificid worms over 90 days (mean±SD).

Treatments	Standing biomass of 90th day (S) $mg \cdot cm^{-2}$	Harvested biomass in 90 days (H) $mg \cdot cm^{-2}$	Total calculated production (S+H) $mg \cdot cm^{-2}$
I	384.07 ± 25.11^{c}	240	624.07 ± 25.11^{c}
II	394.25 ± 41.68^{c}	240	634.25 ± 41.68^{c}
III	825.18 ± 59.28^{a}	240	1065.18 ± 59.28^{a}
IV	501.44 ± 30.72^{b}	240	741.44 ± 30.72^{b}

Values with different Superscripts in a vertical column are significantly different (one way ANOVA followed by Tukey test, $P<0.05$).

During the experimental period temperature, dissolved oxygen and pH of water in culverts under four treatments ranged between 27.0 and 30.3°C, 6.0 and 7.1 ppm, and 7.2 and 7.5 respectively. Only 1.01 kg culture media (20% mustard oil cake, 20% wheat bran, 30% soybean meal, 20% cow-dung and 10% sand soaked with rice gruel) costing US$ 0.29 was needed to yield 1 kg worms.

Discussion

The highest yield of 999.16±40.29 $mg \cdot cm^{-2}$ found at 70th day sampling in treatment-III indicates the suitability of this media to enhance yield compared to the production of three other treatments (502.52±20.68 $mg \cdot cm^{-2}$, 506.49±19.67 $mg \cdot cm^{-2}$ and 674.13±42.18 $mg \cdot cm^{-2}$ in treatment-I, treatment-II and treatment-IV respectively). The present study concluded that the medium soaked with rice gruel is more suitable for culturing tubificid worms than those recommended by Ahamed and Mollah (1992) and Mosharaf (2009) (Table 5). Marian and Pandian (1984) reported a production of 200 $mg \cdot cm^{-2}$ on a substrate containing 75% cow-dung and 25% sand. Ahamed and Mollah (1992) found better production (419.4 $mg \cdot cm^{-2}$) on a medium containing 20% mustard oil cake, 35% wheat bran, 25% cow-dung and 20% sand whereas Mosharaf (2009) reported better production (503.39±22.98 $mg \cdot cm^{-2}$) on a medium containing 35% mustard oil cake, 20% wheat bran, 25% cow-dung and 20% sand. The yield (674.13±42.18 $mg \cdot cm^{-2}$) in treatment-IV has been recorded for the overall effects of soybean meal (30%) used in the experiment. The observed exceptionally better yield (999.16±40.29 $mg \cdot cm^{-2}$) recorded at 70th day culture period in treatment-III clearly indicates the effects of rice gruel on the production of these worms. It also demonstrated the beneficial effects of excess carbohydrate (83.03%) contained in rice gruel on the growth of tubificid worms and suitability of rice gruel to wet the media ingredients instead of water. The lower yield reported in treatment-I and treatment-II might be due to the less suitable culture media used in the experiment.

The peak production of tubificid worms found at 70th day sampling across all the media treatments indicates the suitability of this duration for the ideal carrying capacity of the biomass for optimum propagation of the worms. This might be because of their short generation time i.e., 42 days (Marian and Pandian, 1984). Kaster (1980) stated that 50% tubificids reached sexual maturity within 40 days at the temperature of 15°C on 7% organic carbon content.

Our previous experiments indicate that the harvest level below 40 mg·cm^{-2} leaves the media under-harvested because the initial standing biomass gradually increases ultimately reaching the carrying capacity of the culture system. And just after exceeding the level of carrying capacity of the culture system, the production starts dropping because of sudden death of a considerable number of tubificid worms due to oxygen depletion. The tubificid population usually became distorted after the media introduction because of high decomposition which requires high amount of oxygen in the media. On the other hand, the harvest level above 40 mg·cm^{-2} makes the media over harvested. The biomass was not hampered at a harvest rate of 40 mg·cm^{-2} every 10 days. Therefore, a harvest rate of 40 mg·cm^{-2} at 10 days interval was used during the present experiment.

The study demonstrated that only 1.01 kg media ingredients (20% mustard oil cake, 20% wheat bran, 30% soybean meal, 20% cow-dung and 10% sand soaked with rice gruel) were needed to yield 1 kg worms compared to 18 kg and 25 kg cow-dung as reported by Marian and Pandian (1984) and Marian et al. (1989), respectively. Ahamed and Mollah (1992) needed 2.85 kg media ingredients for producing 1 kg worms whereas Mosharaf (2009) needed 1.99 kg media ingredients to yield a similar quantity of worms. So, from the economic point of view the media (treatment-III) used in the present study proved better for the production of tubificid worms compared to the ones reported by other researchers.

Table 5. Comparison of yield of tubificid worms in different culture conditions.

Culture site	Media used	Standing biomass ($mg\ cm^{-2}$)	Cost per kg worms (US$)	References
Outdoor culvert system	Mustard oil cake, wheat bran, cow-dung and sand	419.4	-	Ahamed and Mollah, 1992
Outdoor culvert system	Mustard oil cake, wheat bran, cow-dung and sand	503.39	0.40	Mosharaf, 2009
Culvert system	Mustard oil cake, wheat bran, soybean meal, cow-dung and sand soaked with rice gruel	999.16	0.29	The present study

Conclusion

Out of four different media used in the experiment, the one comprising 20% mustard oil cake, 20% wheat bran, 30% soybean meal, 20% cow-dung and 10% sand soaked with rice gruel was proved best showing the highest production (999.16±40.29 $mg \cdot cm^{-2}$) of tubificid worms. The success obtained through this work can serve as an important base for future research on this topic.

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