

DEVELOPMENT OF A CULTURE TECHNIQUES FOR TUBIFICID WORM, UNDER LABORATORY CONDITIONS

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Abstract: Study has been conducted to establish a suitable culture technique for Tubificid worm, *Tubifex tubifex* using different culture media. Cowdung, Rawfish and vegetables were evaluated as culture medium. Each medium was supplied as supplementary food at the rate of 250 mg/cm² per week for 145 days. Water temperature, pH and ammonia of the rearing media ranged from 27.1-31.67°C, 5.76-7.35 and 0.5 - <2.5 mg/l respectively. Among the three media, the worms grew well in size in the cowdung medium whereas rawfish showed a moderate growth of worms with remarkable number of new generation of worms. There was no significant growth of worms in the vegetables. The collection of *Tubifex* was quite difficult but highest amount was in cow dung (8.192 mg/g), moderate in rawfish medium (4.14 mg/g) and lowest in the vegetables (2.43 mg/g). Cowdung medium showed the maximum growth in this study period which is more viable, easy and economical.

Key words: Culture techniques, Tubificid worm, Laboratory conditions.

INTRODUCTION

Intensive fish culture in Bangladesh is greatly hampered by crucial curtail of essential feed ingredients. Live feed organisms are performed by most of the cultured larvae compared to artificial feed (Sorgeloos 1979, Immanuel *et al.* 2001). The quality seeds of catfishes are mostly dependent on proper feed management in which production of live foods particularly the tubificid worms in rearing catfish larvae is important (Hossain *et al.* 2011) Live food organisms like *Daphnia* sp. (Marian and Chandran 1989) and *Artemia* (Dwivedi *et al.* 1980, Basil *et al.* 1987; Marian *et al.* 1991, Marian, 1993) have been cultured using organic waste like cowdung, poultry manure, cabbage wastes, coconut mesocarp waste and pig dung in order to reduce the production cost as well as to utilize the waste for food production.

Tubificid, commonly known as sludge worm is one of the cosmopolitan freshwater oligochaetes that plays an important role as supplementary food (live or freeze dried) in aquaculture. This oligochaeta worm, *Tubifex* sp. is one of the best and cheapest live feed for fish, prawns and frogs (Marian and Pandian 1984). It has been reported to be an important live feed in rearing the larvae of

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hatchery produced catfishes, prawns, ornamental fishes etc. (Hossain *et al.* 2011.). Significantly higher survival rates and 10 times additional growth rates were recorded in *C. batrachus* larvae fed with tubificid worms over those formulated dry feed (Alam and Mollah 1988). Tubificid worms are very important to freshwater intensive aquaculture particularly because of their high food values (5575 cal g⁻¹ on a dry weight basis (Giere and Pfannkuche 1982).

In Bangladesh, the current supply of these worms obtained from wild harvests which are unreliable and inadequate in terms of demand. Little success has been reported of attempts taken to develop a technique for tubificid worms culture (Mollah and Ahamed 1989, Ahamed and Mollah 1992). For this reason, it is very important task to develop a compatible culture medium for commercial production of *Tubifex* sp. as an inexpensive alternative to other commercial feeds needed for fish rearing. Therefore, the objectives of the present study were to make a comparative analysis on different culture media and to find out the most suitable food medium for optimal growth of *Tubifex* sp.

MATERIAL AND METHODS

The experiments were conducted over a period of 145 days (from March 2012 to August, 2012) in experimental beds at the Zoology Section, BCSIR Laboratories, Dhaka. At first, Tubificid worms were collected from local drains of BCSIR and cleaned by water flow and held in a water flow-through-system for 24hrs. The worms were cultured in rectangular plastic containers of a particular size of 42 cm x 32 cm x 20 cm with continuous water flow, adjusted through the plastic pipes. Three different types of culture media with three replicates for each treatment were maintained. The media were cowdung (70% cowdung: 30% field soil), rawfish (20% raw fish slice: 80% soil) and vegetables (70% blended vegetables matter of kitchen garbage: 30% soil). The ingredients of all media were mixed with water and kept for 4 days to enhance decomposition. The same media were supplied as supplementary food in each container at the rate of 250 mg/cm² once in every 10 days. Culture media were placed and adjusted 24 hours before inoculation of worms to the media. On the second day, about 1.5g of *tubifex* worms were introduced at the rate of 1.09 mg/cm² and spread homogeneously over the media. About 10g of samples were randomly collected from each media by a sampling spatula (2cm x 2cm) once in every 10 days. After washing, the weights of cleaned worms were weighed through electric balance (Model: AND, GR-200, Japan). Water temperature, pH (Oyster portable pH meter, pp-201, Taiwan), Ammonia (determined by a calorimetric method, APHA, 1985) of the culture media were recorded once in every 3 days. The statistical analysis of different physico-chemical parameters were carried out by using one-

way ANOVA and any difference at 5% level of significance by using the statistical package of SPSS-16 to express the results.

RESULTS AND DISCUSSION

The growth of tubifex worms varied with medium during different experiments conducted (Table 1). The highest average weight of tubifex worms was recorded in cowdung medium having 73.0 ± 56.83 mg/10g whereas medium with vegetables exhibited the lowest weight of 26.35 ± 37.86 mg/10g on an average. Medium with rawfish showed moderate growth rate in case of weight (44.83 ± 33.25 mg/10g) but the highest rate (48.50 ± 26.63 individuals/10g) in case of numbers of new generation. The medium containing vegetables showed lowest rate of growth in numbers of new generation (0.41 ± 0.90 individuals/10g) (Table 1). It is apparent that there was a significant positive relationship ($r= 0.875$) between the numbers of new generation and weight of worms in vegetable culture medium (Tables 2 and 3).

Table 1. Average temperature ($^{\circ}$ C), pH, ammonia (mg/l), weight (mg/10 g) and numbers of new generation of *Tubifex* sp. in different culture media

Culture media	Temperature ($^{\circ}$ C) (Mean \pm SD)	pH (Mean \pm SD)	Ammonia (mg/l) (Mean \pm SD)	Weight (mg/10 g) (Mean \pm SD)	Number of new generation (Indi./10g) (Mean \pm SD)
Cowdung (MC)	29.77 ± 1.3^a	6.92 ± 0.25^a	2.0 ± 0.518^a	73.0 ± 56.83^a	5.33 ± 3.05^b
Raw Fish (MF)	29.30 ± 1.35^a	6.98 ± 0.24^a	1.39 ± 0.40^b	44.83 ± 33.25^{ab}	48.50 ± 26.63^a
Vegetables (MV)	29.42 ± 1.44^a	6.73 ± 0.43^a	1.92 ± 0.64^a	26.35 ± 37.86^b	0.41 ± 0.90^b

Means having the same letters in column do not differ significantly at 5% level of significance.

Table 2. Correlation between weight and pH, temperature, ammonia and number of generations

Culture media	Temperature	pH	Ammonia	Number of new generation
Cowdung	-0.432	0.213	-0.339	0.159
Raw Fish	-0.172	-0.384	-0.025	0.249
Vegetables	0.027	-0.317	-0.160	0.875**

*Significant at the 0.05 level (2-tailed), ** Significant at the 0.01 level (2-tailed).

There was no significant difference among the physico-chemical parameter viz. temperature (F-0.550, $p>0.581$), pH (F-2.224, $p>0.122$) and ammonia (F-5.457 $p>0.008$) of three culture media on growth (F-4.580, $p>0.018$) and number of new generation worms (F-34.203, $p>0.000$). The recorded average temperature and pH were more or less similar but ammonia showed little variation in all the

media. Under study, water temperature ranged from 26.62°C to 31.66°C. There was an insignificance difference between the number of new generation and temperature of the three culture media. The relationship between mean ammonia level and number of new generation worms of cowdung media shows statistically positive relationship at 5% level of significance (Table 3). The average pH values were between 6.73 ± 0.43 to 6.98 ± 0.24 . It was found that pH of the rawfish medium indicated a significant negative relationship ($r=-0.583$) with the growth of new generation worms (Table 3).

Table 3. Relationships between number of new generation and pH, temperature, ammonia, weight

Culture media	Temperature	pH	Ammonia	Weight
Cowdung	0.181	-0.099	0.579*	0.159
Raw Fish	0.491	-0.583*	0.290	0.249
Vegetables	0.177	-0.451	-0.039	0.875**

* Significant at the 0.05 level (2-tailed), ** Significant at the 0.01 level (2-tailed).

Figure 1 shows that the comparative effects of three cultured media on the tubifex throughout the experiment. In cowdung medium, the weight of worms showed remarkable fluctuation of increase and decrease with the higher yield on

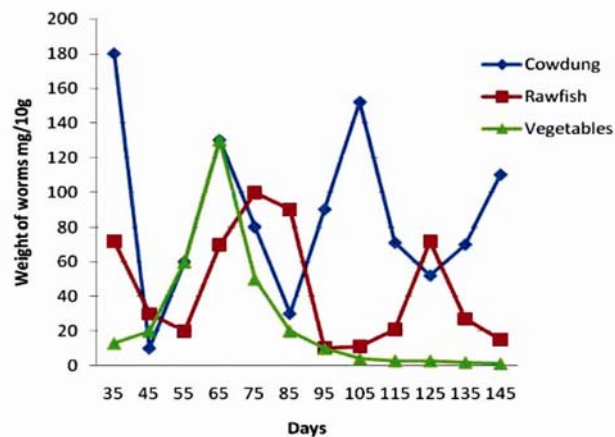


Fig. 1. Effects of culture media on the growth (weight) of *Tubifex* worms under study period.

the 65th and 105th days whereas lowest on the 45th and 85th day's record (Figure 1). In rawfish medium, the weight of worms showed irregular pattern with slight decrease on the 55th day before reaching at optimum growth on the 75th day of sampling and then decreased on the 95th and 105th day with slight increase on the 125th day. The growth of worms in vegetable medium attained a peak on the 65th day and then decreased sharply to the lowest growth rate (1.3 mg/10 g).

The effect of culture media on the number of new generations of *Tubifex* worms is found in Figure 2. The highest growth rate was found in medium having rawfish upto 82 individuals/10g on the 85th day of culture which gradually decreased to 12 individuals/10g. On the other hand, cowdung and vegetable media exhibited more or less similar trends during study period i.e. fewer numbers of progeny produced. The least number (0-1/sample) of offspring was noticed in vegetable based medium.

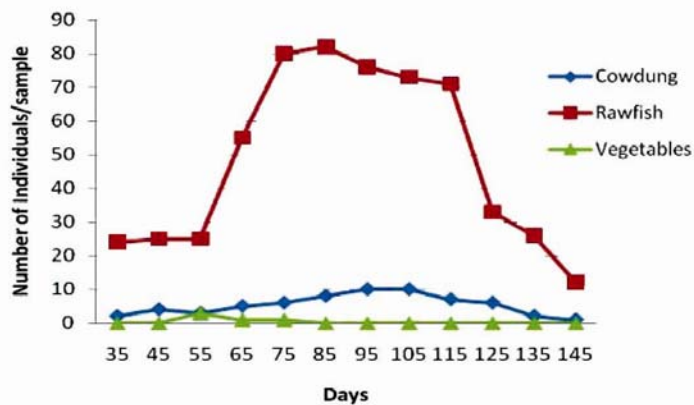


Fig. 2. Effects of culture media on the growth (number of new generation) of *Tubifex* worms.

The observed highest yield 936 mg/120g sample was observed in 145days sampling containing 70% cowdung and 30% sand. The lower yield (316.3 mg/120g sample) in vegetable medium might be because of their less suitability as a culture medium. Marian and Pandian (1984) reported yield of 1g worms using 2.85g media ingredients of 75% cowdung and 25% sand which is conformity with that of cowdung medium.

The temperature of water in culture media ranged from 27.5°C - 31.67°C in cowdung medium; 27.1 °C - 31.66°C in rawfish medium and 26.62°C - 31.66°C in vegetable medium. Randall *et al.* (2011) reported that the optimal culture conditions i.e., temperature, density, and feed type etc were unknown for *T. tubifex*. He recorded the performance of worms fed on cow manure was poor and its growth and recruitment were best when they were supplied with a commercial fish-flake food (Tetramin) or a commercial sinking fish feed having *Spirulina* sp.

Hossain *et al.* (2011) reported that the highest yield of worms (659.35 ± 16.88 mg/cm²) was detected after 70th day of sampling over 80 days duration in optimum culture media containing a mixture of 30% soybean meal, 20% mustard oil-cake and 10% sand in combination with a fixed ratio of 20% wheat bran and 20% cowdung

Therefore, the supply of *Tubifex* worms for fish culture specially aquarium fishes could be increased by using the cowdung medium through large scale production beds. More intensive study is needed for the development of precise method for sustainable production of this worm on commercial range.

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