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Effects of Soybean Meal and Mustard Oil Cake on the Production of Fish Live Food Tubificid Worms in Bangladesh

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Abstract: This experiment was conducted to evaluate the effects of soybean meal and mustard oil cake on the yield of tubificid worms. The highest significant ($p<0.05$) yield 659.35 ± 16.88 mg cm^{-2} of tubificid worms was found in the 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% cow-dung. This highest yield of worms was detected after 70th day sampling over 80 days culture duration. Only 2.50 kg (valued US$ 0.70) of culture media were needed to yield per kg worms. Findings of the present study suggest that the mixture of 30% soybean meal, 20% mustard oil cake, 20% wheat bran, 20% cow-dung and 10% sand is the best culture media for a period of 70 days culture duration to get the maximum production of tubificid worms.

Key words: Bangladesh · Live food · Tubificid worms · Soybean meal and mustard oil cake

INTRODUCTION

In Bangladesh, nearly 40% of total country's fish yield comes from aquaculture which mostly depends on hatchery produced fish seeds. Where as, catfishes contribute nearly 3.4% of total inland fishery production [1]. The farming of stinging catfish *Heteropneustes fossilis*, walking catfish *Clarias batrachus*, pabda *Ompok* spp., pangasius catfish *Pangasius hypophthalmus* etc. is becoming progressively more popular in Bangladesh. Production and reliable supply of good quality fingerlings in sufficient quantity is essential to sustain this section of the aquaculture industry. The quality seeds of catfishes are mostly depend on proper feed management in which production of live foods particularly the tubificid worms in rearing catfish larvae is important. The mass production of live foods for example, tubificid worms in nursing and rearing the catfish larvae and ornamental fishes may be a way out of the problems facing by the hatchery owners and nursery growers.

In aquaculture, feed is the single most important item since nearly 60% cost is associated with fish feed. Tubificid worms are very important to fresh water intensive aquaculture particularly because of their high food values (5575 cal g^{-1} on a dry weight basis) [2]. These worms have already been used to support commercial fish farming in the USSR [3]. On the other hand, these worms are also used throughout the world, including Bangladesh, as food for aquarium fishes. Tubificid worms have been reported to be an important live food in rearing the larvae of many commercially important fishes particularly the catfishes. Significantly higher survival rates and 10 times additional growth rate was recorded in *C. batrachus* larvae fed tubificid worms over those formulated dry feed [4].

They grow in a place with steady and continuous water flow with high organic detritus. In Bangladesh, the current total supply of these worms comes from wild harvests which are unreliable and inadequate in terms of demand. Harvest from the wild is hazardous to collect for unhealthy conditions. Information related to culture of tubificid worms in Bangladesh is meager. Little success has been reported of several attempts taken to develop a technique to culture tubificid worms [5, 6].

Thus, there is a need to develop a technique suitable to get reliable supply meeting the growing demand. Therefore, the present study was undertaken with a view to determine the effects of soybean meal and mustard oil cake on the yield of tubificid worms.

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MATERIALS AND METHODS

Experimental Worms and System: Tubificid worms were collected from different places of Bangladesh. The collected worms were cleaned and held in a flow-through-system for conditioning over 24 hours before inoculation. The experiment was conducted from April to June 2010 for 80 days. The worms were cultured in indoor cemented culverts (160 x 25 x 10 cm^3) system to protect from rain and sunlight or any other natural hazards. Before beginning the experiment, the culverts were washed and cleaned with fresh water. The culverts were connected with a flow-through-system in which subsurface well water was used. Each culvert was given continuous water spray by using a horizontal porous PVC pipe (180 cm long and 1 cm^2 diameter). Experiment was conducted in the Department of Fisheries Biology and Genetics, Bangladesh Agricultural University (BAU).

Experimental Design: A 3 × 3 × 5 factorial design was applied in triplicate group. The experimental diets were tested, respectively, three levels (10, 20 and 30%) of soybean meal (SM) against three levels (10, 20 and 30%) of mustard oil cake (MOC) with five sampling durations (40, 50, 60, 70 and 80 days). In this experiment, 20% wheat bran (WB) and 20% cow-dung (CD) were also used in fixed ratio. In addition to these media ingredients, sand was used only as substrate to settle the media into culture culverts and to make the media mixture 100% (Table 1).

Culture Media: Required amount of media ingredients was measured by a laboratory balance on proportional basis to make up 1kg of media for each culvert and mixed thoroughly by hand with sufficient amount of water in plastic bowl. The wet mixture of media ingredients was held in this form for seven days for better decomposition. After seven days, the well prepared media was placed into the culture culverts.

Inoculation of Tubificid Worms: Water flow adjusted 24 hours before inoculation of worms to the culverts. The collected tubificid worms were inoculated at the rate of 2.5 mg cm^{-2} (i.e. 10 g $culvert^{-1}$). They were spread over the media homogeneously as much as possible in each of the culvert.

Periodic Supply of Culture Media: The periodic supply of culture media was done after 10 days of worms inoculation. The prepared media were introduced at the rate of 250 mg cm^{-2} in respective culverts once in every 10 days at 10 a.m. before sampling. Total quantity of media was spread throughout the culverts. At that time, water flow was stopped for a while.

Water Quality: Water flow rate (L min^{-1}) was measured once in 10 days with the help of measuring cylinder. Continuous water flow was maintained which was able to keep the dissolved oxygen in suitable range (4-6 mg L^{-1}). Water temperature (°C) of the culture culverts and dissolved oxygen (mg L^{-1}) was detected with the help of a portable dissolved oxygen meter (Jenway, Model No. 9070, UK) before sampling at 10.00 a.m. once in every 10 days.

Sampling: Samples were drawn at 40, 50, 60, 70 and 80 day after inoculation of worm. Tubificid worms were collected by a sampler (4.4 × 4.4 cm^2) with water and media from five randomly selected places of each culvert. They 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 blotting paper and weighted by Mettler Electric balance (Switzerland) graduated in 0.000 g.

Estimation of Media and Production Cost: Media requirement and per kilogram cost of worms in Bangladeshi Taka (BDT) were determined. The cost in BDT is then converted into US dollar (1 US$ = 70 BDT).

Table 1: Media ingredients used in the experimental diets

Culture media ingredients	Levels of media ingredients (%)								
Soybean meal	10			20			30		
Mustard oil cake	10	20	30	10	20	30	10	20	30
Wheat bran	20	20	20	20	20	20	20	20	20
Cow-dung	20	20	20	20	20	20	20	20	20
Sand	40	30	20	30	20	10	20	10	0
Total (%)	100	100	100	100	100	100	100	100	100

Statistical Analysis: Data were analyzed by using ANOVA followed by Tukey's HSD post hoc for multiple comparisons. Data have been presented as mean ± SEM and analyzed by using the statistical software SPSS version 11.5 with the level of significance at $p<0.05$.

RESULTS

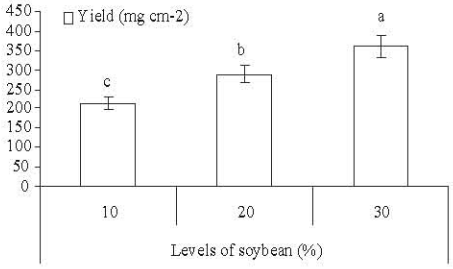
Effects of soybean meal (SM) and mustard oil cake (MOC) on the yield of tubificid worms for five sampling duration.

Overall effects of SM: Culture medium containing 30% soybean meal (SM) yielded the highest harvest (358.81 ± 28.46 mg cm^{-2}) that was 1.68-folds higher than did the medium with 10% SM (213.74 ± 16.86 mg cm^{-2}; Figure 1a). However, harvest from the medium with 20% SM (289.31 ± 22.11 mg cm^{-2}) was 1.35-times higher than that of the medium with 10% SM.

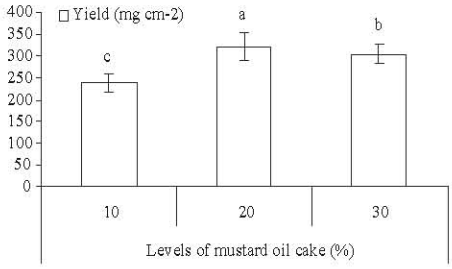
Overall effects of MOC: The culture medium with 20% MOC had the highest yield of tubificid worms (320.38 ± 30.18 mg cm^{-2}) which was 1.34-folds higher than in the medium having 10% MOC (238.97 ± 20.17 mg cm^{-2}; Fig. 1b). However, 30% MOC yielded 1.27-times higher worms (302.52 ± 22.42 mg cm^{-2}) than that of 10%.

Overall Effects of Durations: Regardless the culture mediums, 70 day sampling had the highest yield of tubificid worms (421.73 ± 26.27 mg cm^{-2}) that was 3.57-folds higher than did the 40 day sampling (Fig. 1c). Harvests of the 80 (363.00 ± 25.72 mg cm^{-2}) and 60 (309.28 ± 21.33 mg cm^{-2}) day samplings, respectively, were 3.08- and 2.62-times higher than that of 40 day sampling. Sampling of 50 day (224.44 ± 17.69 mg cm^{-2}) was also 1.90-folds higher than in the first sampling.

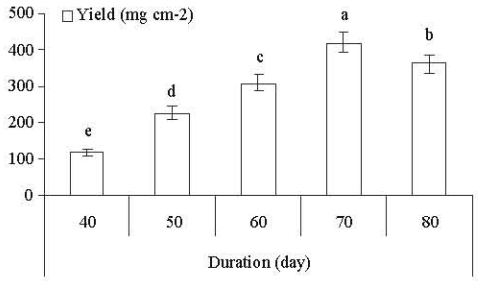
Effects of 10% SM with All Possible Combination of 10, 20 and 30% MOC for Five Sampling Durations: The more suitable yield was observed in the culture media having 10% SM and 30% MOC. However, in all three treatments of MOC having 10% SM in the culture media, 70 day sampling had the highest yield (10% MOC: 299.23 ± 23.85 mg cm^{-2}; 20% MOC: 336.17 ± 34.11 mg cm^{-2}; 30% MOC: 357.79 ± 15.60 mg cm^{-2}) whereas the lowest (76.13 ± 5.87 mg cm^{-2}; 10% MOC: 86.12 ± 3.66 mg cm^{-2}; 20% MOC 104.50 ± 11.63 mg cm^{-2}; 30% MOC) was found in 40 day sampling (Figures 2a-c).The yields of 10 and 20% MOC at 70 day were similar to 80 day and 60 day sampling. On the other hand, the yields of 60 day were similar to 50 day but different from 40 day sampling. In addition, the trend of yield at 30% MOC was different from 10 and 20% MOC where 50 and 60 day sampling were similar.



(a) Overall effects of SM



(b) Overall effects of MOC



(c) Overall effects of duration

Fig. 1(a-c): Yield of tubificid worms harvested from different mediums with all possible combinations of 10, 20 and 30% soybean meal and mustard oil cake for a period of 40, 50, 60, 70 and 80 days. Bars (± SEM) with different letters are significantly different (ANOVA, HSD, $p<0.05$).

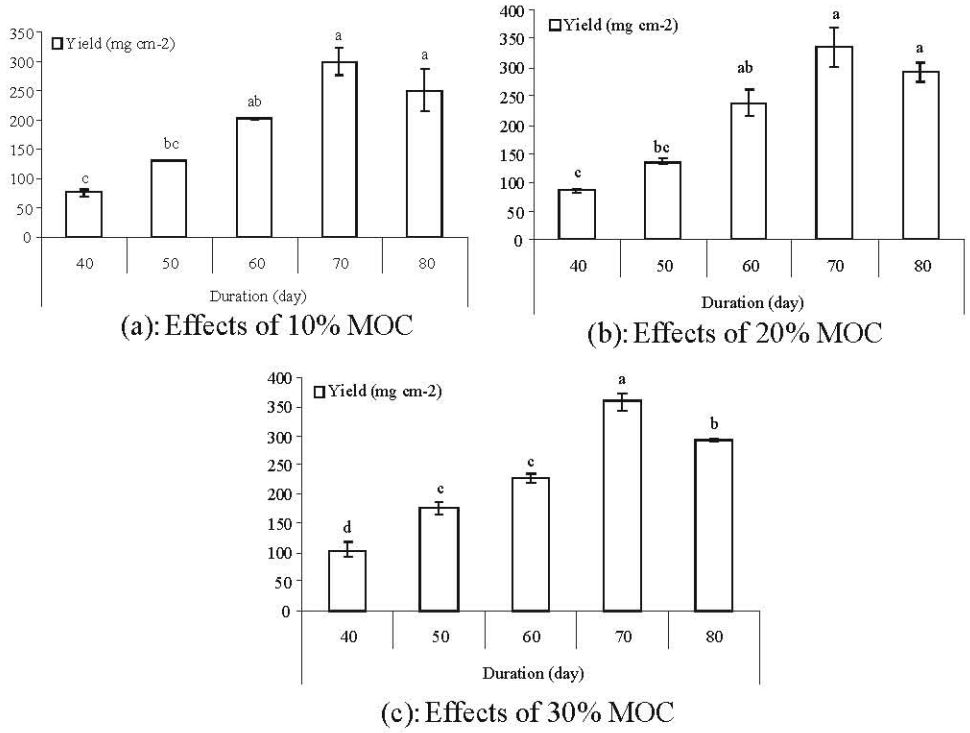


Fig. 2(a-c): Tubificid worms yield (mg cm^{-2}), harvested from 10% SM with 10, 20 and 30% MOC over 40, 50, 60, 70 and 80 day culture period. Bars (± SEM) with different super script letters indicate significant differences (ANOVA, HSD; $p<0.05$).

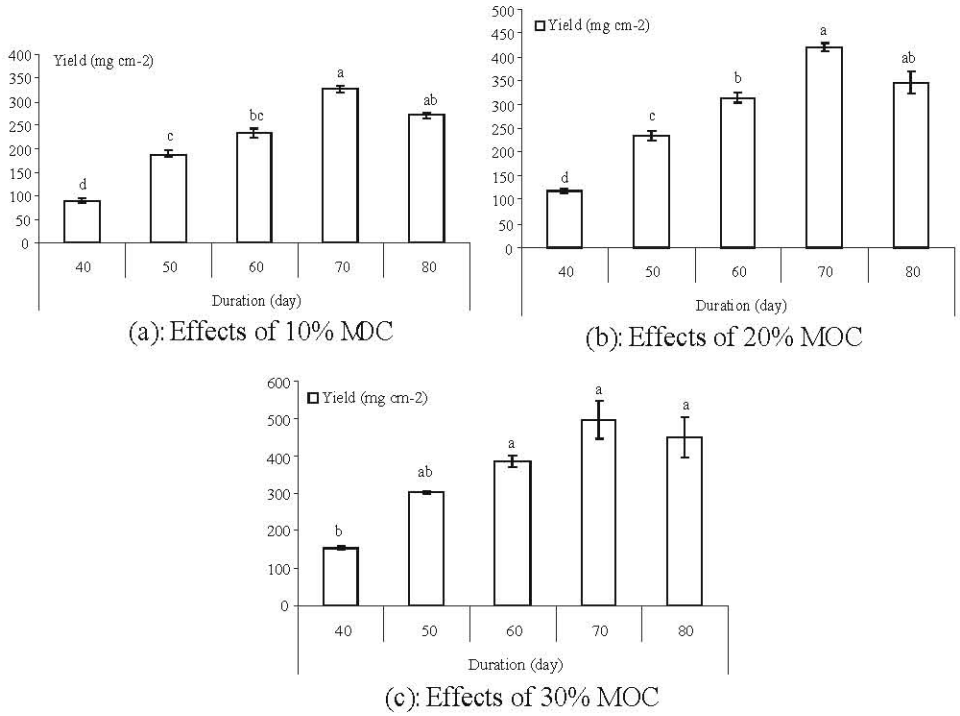


Fig. 3(a-c): Tubificid worms yield (mg cm^{-2}), harvested from 20% SM with 10, 20 and 30% MOC over 40, 50, 60, 70 and 80 day culture period. Bars (± SEM) with different super script letters indicate significant differences (ANOVA, HSD; $p<0.05$).

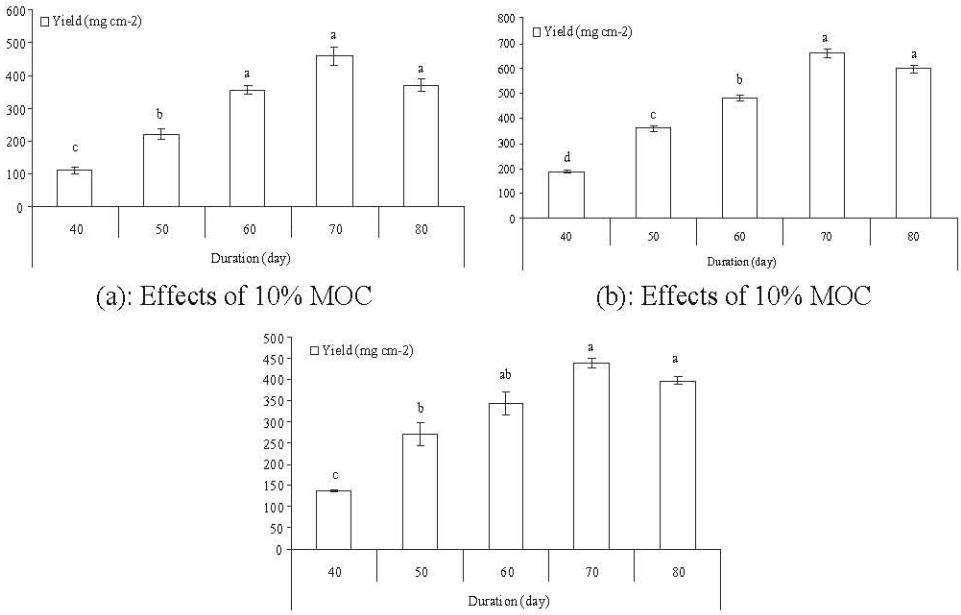


Fig. 4(a-c): Tubificid worms yield (mg cm^{-2}), harvested from 30% SM with 10, 20 and 30% MOC over 40, 50, 60, 70 and 80 day culture period. Bars (± SEM) with different super script letters indicate significant differences (ANOVA, HSD; $p<0.05$).

Effects of 20% SM with All Possible Combination of 10, 20 and 30% MOC for Five Sampling Durations: Tubificid worms in three treatments, MOC having 10, 20 and 30% whereas 20% SM in the culture media, at 70 day sampling observed the highest yield (10% MOC: 327.94 ± 24.51 mg cm^{-2}; 20% MOC: 421.01 ± 8.79 mg cm^{-2}; 30% MOC: 498.14 ± 51.03 mg cm^{-2}) while the lowest (90.53 ± 5.32 mg cm^{-2}; 10% MOC: 118.28 ± 4.98 mg cm^{-2}; 20% MOC: 154.94 ± 5.16 mg cm^{-2}; 30% MOC) was found in 40 day sampling (Fig. 3(a-c)). At 10% MOC, the yields of 70 day were similar to 80 day but different than that of 60 day. On the other hand, the yields of 80 day were similar to 60 day but different from 50 day sampling and 60 day yield were similar to 50 day and the other was different. The yield of 70 day was similar to that of 80 day but different from 60 day sampling at 20% MOC, while the yield of 80 day was similar to that of 60 day sampling. Sampling of 40-60 day was significantly different. In the culture medium with 30% MOC, although the yields of 50-80 day sampling similar but 50 day sampling was different from those of 70 and 80 days. However, no difference was found in the yield sampled between the day of 40 and 50. However, the maximum yield was observed in the culture media having 20% SM and 30% MOC in this media treatment.

Effects of 30% Soybean Meal with All Possible Combination of 10, 20 and 30% Mustard Oil Cake on the Yield of Tubificid Worms for Five Culture Durations: In all three media treatments combining 10, 20 and 30% MOC with 30% SM, 70 day sampling had the highest yield (10% MOC: 457.76 ± 29.48 mg cm^{-2}; 20% MOC: 659.35 ± 16.88 mg cm^{-2}; 30% MOC: 438.17 ± 10.95 mg cm^{-2}) whereas the lowest (110.72 ± 9.60 mg cm^{-2}; 10% MOC: 184.15 ± 5.18 mg cm^{-2}; 20% MOC: 136.72 ± 3.43 mg cm^{-2}; 30% MOC) was found in 40 day sampling (Fig. 4; a-c). At 10% MOC, the yield of 60, 70 and 80 day sampling had the similar trends except 40 and 50 day sampling. The yield of 20% MOC at 70 and 80 day was similar but 40, 50 and 60 day sampling was significantly different. In addition, the yield of 30% MOC at 80 day was similar to 60 day but not 50 day sampling. The yield at 40 and 50 day was significantly different. However, the maximum yield (659.35 ± 16.88 mg cm^{-2}) was observed in this culture media having 30% SM and 20% MOC that was the best culture media in this experiment for tubificid worms culture.

Production Cost: Only 2.65 kg culture media valued BDT 60 (20% WB, 30% SM, 20% MOC, 20% CD and 10% sand) was required to yield 1 kg of worms.

Water Quality: Water temperature of culture water was ranged from 23-27 °C throughout the entire experimental period. Water flow (1.23 ± 0.33 L min^{-1}) was maintained which was able to keep the dissolved oxygen between 4 and 6 mg L^{-1}.

DISCUSSION

The observed highest yield 659.35 mg cm^{-2} has been found at a 70 day sampling in the culture medium containing 30% SM, 20% MOC, 20% WB, 20% CD and 10% sand across all treatments in this experiment indicates the suitability of this medium to enhance yield compared to the previous yield (419.4 mg cm^{-2}) as demonstrated elsewhere [6]. The lower yield reported in the previous study in a medium of 35 % WB, 25% CD, 20% MOC and 20 % sand might be because of their less suitability as culture medium. In addition, the present study has been demonstrated that only 2.65 g media ingredients (20% WB, 30% SM, 20% MOC, 20% CD and 10% sand) were needed to yield 1 g worms while 18 g and 25 g media ingredients of 75% CD and 25% sand and 2.85 g media ingredients were reported to yield the similar quantity of worms [6-8].

The observed overall effects of SM have been found the highest yield (358.81 mg cm^{-2}) of tubificid worms in the culture medium containing 30% SM but the lowest yield (213.74 mg cm^{-2}) in the medium having 10% SM. On the other hand, 20% SM yielded (289.31mg cm^{-2}) better yield than that of 10% SM. The 30% SM is a suitable media ingredient for the preparation of tubificid culture medium. Tubificid worms may be utilized the maximum nutrient from this medium.

The better yields (320.38 mg cm^{-2}) have been recorded for the overall effects of MOC while the medium having 20% MOC denotes the suitability of MOC for the preparation of culture medium. The authors were found the similar quantity of MOC for the better growth and survival of tubificid worms [7].

The highest production (421.73 mg cm^{-2}) of tubificid worms has been found for the overall effects of duration at a 70 day sampling across all the media treatments indicates the suitability of this duration for the maximum carrying capacity of the biomass by optimum propagation of worms. However, durations before and after 70 day may not be suitable for maximum production and growth of the tubificid worms in these media treatments.

This experiment demonstrated that WB, SM and MOC are important media ingredients and had a definite effect on the culture of tubificid worms. These ingredients contained valuable minerals, proteins, organic matter etc. [7]. The medium (20% WB, 30% SM, 20% MOC, 20% CD and 10% sand) was the best because it contained sufficient amount of organic carbon, minerals, proteins, vitamins etc.. Organic carbon was the essential part for the reproduction of tubificid worms. 50% tubificids reached sexual maturity within 40 days at 15 °C temperature on 7% organic carbon content [10]. He also stated time requirement for reaching sexual maturity significantly decreased when temperature and organic carbon content in the culture media was increased.

The 2.65 kg culture media (20% WB, 30% SM, 20% MOC, 20% CD and 10% sand) required yielding per kg worms. The physico-chemical factors such as water flow, water temperature and dissolved oxygen of the culture water content have been recorded during the period of study were suitable and productive range as found [7, 10, 11]. The observed temperature of culture water ranged from 23 °C to 27 °C throughout the entire experimental period. 11 °C is the minimum temperature for the reproduction of *Tubifex tubifex* while 2.5 °C and 38 °C are the lethal [12]. Dissolved oxygen contents of culture water depend on water flow (1.23 ± 0.33Lmin^{-1}) which able to keep the dissolved oxygen 4-6 mg L^{-1}. This range of DO was suitable for the culture of tubificid worms.

The results of this study have shown that media ingredients containing 30% Soybean meal, 20% Mustard oil cake, 20% Wheat bran, 20% Cow-dung and 10% sand may be used in yielding 659.35 mg cm^{-2} tubificid worms over 70 days culture duration.

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