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Comparative Study of Growth and Reproduction of Earthworm Eudrilus Eugeniae in Different Organic Substrate

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ABSTRACT

Utilization of Bio-methanated Distillery Effluent (Spent Wash) as a potential substrate in vermiculture of Eudrilus eugeniae was studied at Advanced Research Farm Facility (ARFF) of Tamil Nadu Fisheries University, Madhavaram campus. The biological parameters of earthworm Eudrilus eugeniae were recorded when reared in different vermibeds treated with domestic waste (DW), crude spent wash (CSW) and diluted spent wash (DSW). Cow dung (CD) and leaf litter (LL) were used as base substrate material in all treatments. The experiment was conducted in perforated trays (45 x 30 x 10 cm) for a period of 10 weeks, in duplicates. Four number of non-clitellated adults (950-1025mg live weight) were introduced in each experimental unit. The experiment was conducted at an ambient temperature of 28-30°C. Growth parameters such as mean individual live weight, maximum individual growth rate (mg/worm/day), total biomass gain; reproductive performance indicators such as number of cocoons, reproduction rate (cocoon/worm/day) and hatching viability were significantly higher in vermibed treated with diluted spent wash. Mortality and growth retardation was observed in the vermibed treated with crude spent wash. To conclude, appropriately diluted spent wash can be used as a potential substrate in the vermiculture operation of Eudrilus eugeniae.

Key words: Spent wash; Vermiculture; African night crawler; Earthworm; Reproduction.

Introduction

One of the most important environmental problems faced by the world is management of industrial wastes. Distillery industries generate enormous amount of waste in the form of spent wash which is the residual liquid generated during alcohol production. In a developing country like India, distillery industries thus have become a major source of pollution, as 88% of its raw materials are converted into waste and discharged into the water bodies (Suthar and Singh 2008), causing water pollution. Around 212 distillery units in India generate more than 30 billion liters of spent wash annually. The spent washes released of are major source of soil and aquatic pollution due to presence of water-soluble recalcitrant colouring compounds called melanoidins. Melanoidins are very important from environmental aspects and due to their structural complexity, dark colour and offensive odour; they pose serious threat to soil and aquatic ecosystem. It also cause reduction of sunlight penetration, decreased photosynthetic activity, depletion of dissolved oxygen concentration, discoloration in the water bodies, reduction of pH(Chhonkar et al,2000) whereas on land, it causes reduction in soil alkalinity and inhibition of seed germination.(Nikham *et al*, 2014). The most important characteristic of spent wash is that it is strongly acidic, dark brown colored hydrophilic viscous liquid waste with strong objectionable odour, highly organic with high BOD and COD values (Rath *et al* 2011). Thus efficient management of this spent wash is the need of the hour for the future of both mother earth and humanity. Recent studies have shown possibilities of using decomposer organism mainly earth worms to process spent wash by cleaner technology such as vermiculture thus minimizing environmental pollution. These earthworms are called as ecosystem bioengineers which has multifarious role to play in a developing country like India. African night crawler, *Eudrilus eugeniae* is used extensively in commercial vermiculture. Increased attention has been given to the species as a possible waste decomposer and as a protein source. Very little work on its ability to act as an efficient decomposer using crude spentwash is done and in order to clearly establish the role of this species as a waste decomposer more study on its biological capability has to be carried out. Hence the present study aims at examination of the growth, fecundity (cocoon production), biomass production, viability of hatchlings of *Eudrilus eugeniae* in substrate treated with spent wash as a major component.

Materials and methods

Experimental animals

The experimental animals used in the present study were African night crawler (*Eudrilus eugeniae*). About 12-16 week old, non clitellated worms weighing in the range of 950±1025 mg were obtained from a commercial vermiculture farm. The worms were transferred in wet gunny material with sufficient moisture from the farm to the vermiculture experimental research unit at Advanced Research Farm Facility (ARFF) of Tamil Nadu Fisheries University, Madhavaram campus.

Preparation of vermi-bed
The leaf litter of *Albizia saman*, a nitrogen fixer in soil and a species of flowering tree in the pea family, Fabaceae, was collected from ARFF campus, Madhavaram. Collected leaves were finely chopped and allowed for partial decomposition for 14 days by sprinkling water periodically. Later it was mixed with dried cowdung at the ratio of 1:1.

Collection of spent wash
Spent wash was collected from the refineries of M/s Rajshree Biosolutions private Ltd from Thiruvanamalai and stored at room temperature. The characterisation of the spent wash for its chemical constituents was done to determine the pH, electrical conductivity, COD, BOD, nitrogen, phosphate, calcium, magnesium, potassium and sodium according to standard methods (APHA 2012).

Experimental design
The experiment was designed to examine the performance of three different combinations of substrate materials in comparison to a control. The widely used substrate combination of cow dung (CD), leaf litter (LL) was used as the base material for all the treatments including control. The control group was added with damp soil in addition to CD and LL. The other treatments namely T1, T2 and T3 were added with the domestic waste (DW), crude spent wash (CSW) and diluted spent wash (DSW) (1:3in water) in addition to the base compost material of CD and LL.

Experimental procedure
The experiment was conducted in perforated trays (45 x 30 x 10 cm) for a period of 10 weeks, in duplicates. The moisture content was maintained at 70-80% during the study period (10 weeks) by sprinkling tap-water in control and all the treatment, on every alternate day. The substrate bed was turned over every day in order to eliminate obnoxious gas, initiation of microbial degradation and softening of waste for initial 7 days, before introducing the worms. Four number of non-clitellated adults (950-1025mg live weight) were introduced in each experimental unit. The trays were placed in a shady area avoiding direct sunlight at an ambient temperature of 28-30◦C. All the containers were covered with moist gunny material to prevent the earthworms from escaping. Cocoon production and biomass gain were recorded weekly for 10 weeks. The produced cocoons were separated from the vermin-bed manually, sorted and counted. Further the cocoons were incubated in respective beds for further development. Production of cocoons was observed every 24 hours. Hatchlings produced were recorded, removed and stocked in separate beds. Since mortality of earthworms was more in T2 and cocoon production almost negligible, T2 treatment was discarded.

Data analysis
The data were subjected to one-way ANOVA to estimate the mean effects of the treatments at a minimum significance level of $p<0.05$. The data were also subjected to Duncan's multiple comparison tests following ANOVA. All the statistical analysis was performed in SPSS v16.

Results

Chemical and nutrient composition of spent wash
The results of the chemical and elemental composition of the crude spent wash and diluted spent wash used in the study is presented in Table 1. The parameters such as pH, colour, COD, BOD, nitrogen, phosphate, calcium, magnesium, potassium and sodium concentrations were analysed and reported.

Table 1: Nutrient content of spent wash

Parameters in mg/L	Results	
	Crude spent wash	Diluted spent wash
pH @ 25 C	4.25	6.9
Total Suspended Solids	42730	9822.4

Organic Solids	252340	52032
Inorganic Solids	74640	15300
Total Phosphorus	66.6	31.7
Calcium	6072.1	1336.5
Magnesium	4421.4	986.2
Potassium	23055	4122.7
Total Organic Carbon	74250	4013
Total Nitrogen	834	286
Lignin	98	39
Cellulose	153	73
Hemicellulose	26	11

Growth performance

The growth curve of the earthworms over the experimental period of 10 weeks is presented in Figure 1. The results showed that substrate treated with diluted (1:3) spent wash (DSW) had the maximum weight gain followed by substrate treated with domestic waste (DW). The growth parameters as assessed by increase in individual weight, total biomass and growth rate per day are presented in Table 2. These growth parameters also showed significantly higher response in DSW(T3) treatment. The earthworms subjected to substrate treated with crude spent wash (CSW) exhibited mortality and retarded growth and hence discarded.

Figure 1: Growth curve of the earthworms over the experimental period of 10 weeks

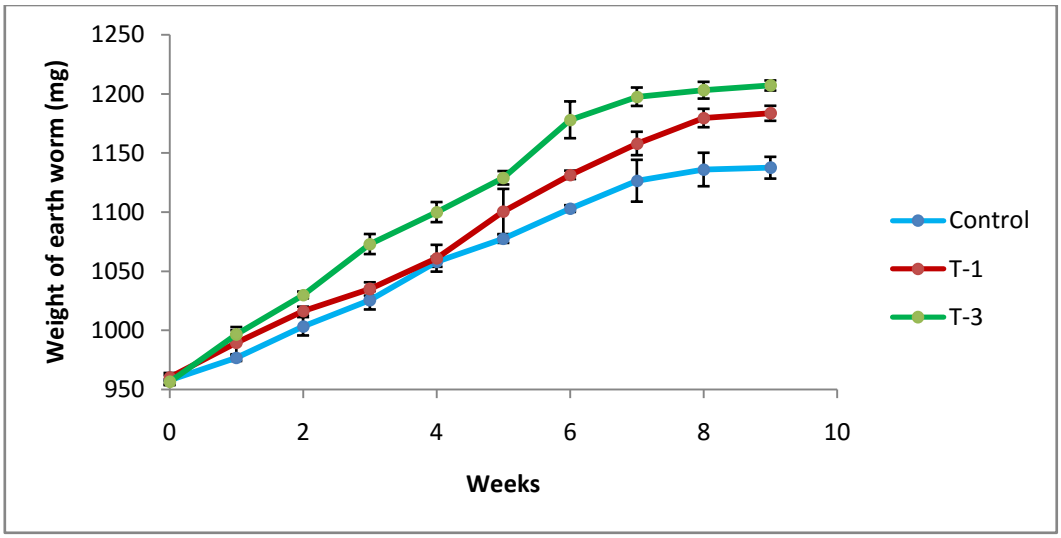

Table 2: Growth performance of *Eudrilus eugeniae* in different substrate treatments

	Control	T-1 (DW)	T-2 (CSW)	T-3 (DSW)	P-value
Initial weight (mg)	957.5 ± 3.54	961.5 ± 4.95	959.5 ± 2.12	961.5 ± 4.95	> 0.05
Final weight (mg)	1137.5 ± 9.2 b	1183.5 ±6.36 c	1006 ± 15.56 a	1207 ± 4.24 c	< 0.01
Weight gain (mg)	180 ± 12.73 b	222 ± 11.31 bc	46.5 ± 13.44 a	245.5 ± 9.19 c	< 0.01
Growth rate (mg/worm/day)	2.57 ± 0.18 b	3.17 ± 0.16 bc	0.66 ± 0.19 a	3.51 ± 0.13 c	<0.01

Reproductive performance

The reproductive performance of earthworms was assessed by monitoring the parameters related to quantitative and qualitative indicators of cocoon and hatchling production.as performed by Reinecke andViijoen (1988) The weekly production of cocoons over the experimental period is presented in Figure 2. Data on the reproductive performance indices are presented in Table 3. The results show that, the production of cocoons was similar between the substrates treated with Domestic waste and Diluted spent wash but were significantly higher (P<0.01) when compared to control. The number of viable cocoons and the hatching percentage were significantly higher in the DSW (T3) treatment (P<0.01) compared to control and DW(T1). The average number of hatchlings per cocoon was not significant (>0.05) was not affected by the treatments.

Figure 2: Weekly production of cocoons over the experimental period

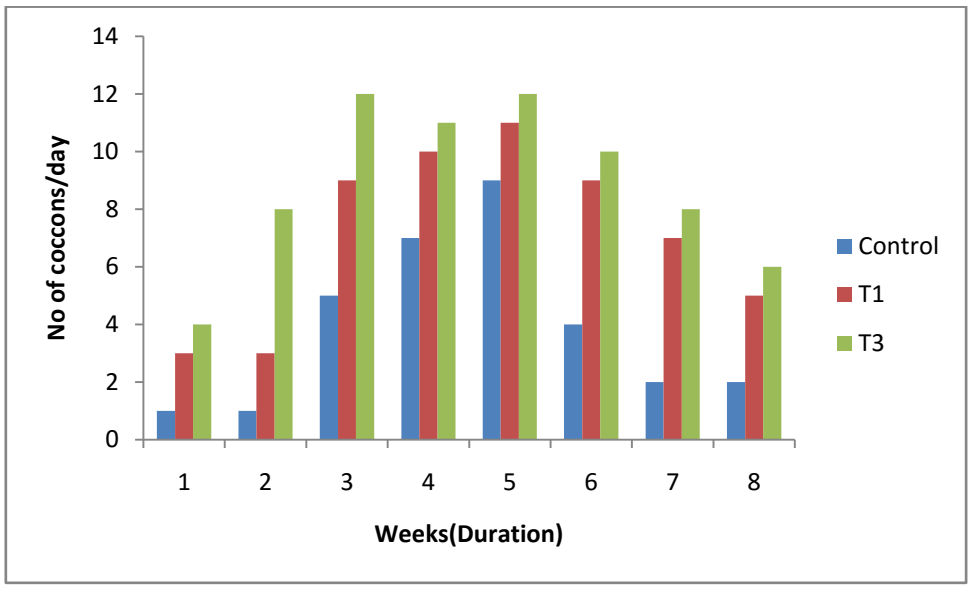

Table 3: Reproductive performance of *E. eugeniae* in different substrate treatments

	Control	T-1 (DW)	T-2 (CSW)	T-3 (DSW)	P-value
Cocoons	39.5 ± 2.12 [b]	66.5 ± 3.54 [c]	4 ± 1.41 [a]	72 ± 1.41 [c]	<0.01
Viable cocoons	27.5 ± 2.12 [a]	53.5 ± 2.12 [b]	NA	67 ± 1.41 [c]	<0.01
Hatchlings	57.5 ± 4.95 [a]	112 ± 4.24 [b]	NA	129 ± 8.49 [c]	<0.01
cocoon/day	0.56 ± 0.03 [b]	0.95 ± 0.05 [c]	0.06 ± 0.02 [a]	1.03 ± 0.02 [c]	<0.01
hatching %	69.58 ± 1.63 [a]	80.48 ± 1.09 [b]	NA	93.05 ± 0.14 [c]	<0.01
hatchling/cocoon	1.45 ± 0.05	1.69 ± 0.15	NA	1.79 ± 0.15	>0.05

Discussion

Table 1 shows the chemical characteristic of all the substrate material used for vermibed. The Data clearly indicate that spent wash possess very low pH value, high electrical conductivity and high organic load. The quality of an organic waste is stated by its physico- chemical parameters, the rearing environmental factors viz. temperature, moisture content and pH determines the onset and rate of reproduction of earthworms (Parthasarathy *et al* 2014). Earthworms are very sensitive to low pH

and high electrical conductivity that limits the growth and survival of earthworms (Shanmugapriya and Lakshmi priya 2011, Senthil Kumar et al 2013 SaiKeerthika *et al* 2015). In the present study pH was too low and electrical conductivity was high in crude spent wash thus showing a decrease in survival of earthworms in T2. Vermiculture is considered in terms of production of earthworm biomass, number of cocoons and its hatching viability using various organic substrates. Murchie (1960) proved experimentally the existence of a significant relationship between weight increase and susbtrate type, which may reasonably be attributed to nutritional quality of the subtrate. In the present study more thrust have been given to the biological triats of the earthworms to ascertain the feasibility of using spent wash as a substrate media. The spent wash which has been used possess higher organic carbon, total phosphorus and total nitrogen and are the requisite for growth and reproduction in earthworms (Soniya and Dhanasekaran 2015). Studies of Garg et al (2005), and Jaweira Siddique et al (2005) have shown higher C:N ratio and phosphorus (2.5%) content in organic waste (press mud)to support better growth (length and biomass) and bring about earlier maturation, earlier differentiation of the clitellum, and release of cocoons in *L. mauritii* and *E. eugeniae* than worms fed with cowdung or clay loam soil alone. In the present study, *E. Eugeniae* exhibited highest biomass, more cocoon, highest hatching viability in T3, followed by T1 and control.

The growth curves of *E. Eugeniae* observed in the different treatments over the observation period are given in Fig 1. Maximum worm biomass was attained in T3 and minimum in control. There was a steady increase in weight by earthworms in T1 and T3 when compared to that of Control. Initially worms gained biomass but later after few weeks, weight loss by earthworms was observed in all the tested waste substrate. The loss in worm biomass can be attributed to the exhaustion of food. When *E. Eugeniae* received food below a maintenance level, there was slow loss of weight at a rate which depended upon the quantity and nature of its ingestible substrates. This is in accordance with the findings of Parthasarathy, 2007 in *E. eugeniae*, who reported the decrease in worm biomass due to the requirement of large amount of energy for cocoon production and also for copulation and the last phase of decrease in worm biomass due to continued reproduction and aging.

The weight gain for *E. Eugeniae* was highest in T3 followed by T1 and in control. Edwards et al. have reported a biomass gain of 292 mg cattle waste by *Perionyx excavatus* at 25 °C. The current study also showed a similar trend in weight gain. The growth rate (mg weight gained/day/earthworm) has been considered a good comparative index to compare the growth of earthworms in different wastes. The substrate treated with crude spent wash supported the least growth of *E.euginea* and the substrate with cow dung and leaf litter were marginally better than domestic waste and diluted spent wash substrate (Table 2). Earthworms attained a peak growth after a certain duration which followed by a slow decrease in their growth at the end of the experiment. Such a trend of weight loss was observed by Sudhar and Singh 2007, in *E.Foetida.* It can be correlated that most of the substrate was converted to vermicompost which cannot further support their growth. Mortality and lower growth of earthworms was observed only in T2 in the 2^{nd} week due to the very low pH, high conductivity of crude spent wash and the higher viscous nature of the spent wash as such. The earthworm's survival and growth depend on the physical and chemical profile of the feed stuff used in the substrate. Since the earthworms are sensitive to pH, it was maintained at a pH above 6.9 -7.1 by turning up the bed periodically to remove obnoxious gases and thus avoid decrease of pH.

The reasons for the enhanced growth and reproduction in T1 and T3 treatments followed by control in the present study can also be attributed to the presence of cellulose, hemicellulose, lignin content and enhanced water holding capacity (43-51%) which enables the T3 treatment to maintain good and ideal moisture. This was also been stated by Parthasarathy et al, 2014, Soniya and Dhanasekaran 2015 et al in a study conducted using leaf litter and press mud as substrate material. The dependency of earthworm on soil moisture for their survival and activity and on organic matter rich in Nitrogen for growth and reproduction is well known (Revathi et al 2014). The leaf litter which was used in the current study belong to pea family which is rich in Nitrogen content was an added benefit in the substrate to boost the growth and reproduction. The T3 treatment provides such ideal physico-chemical conditions suitable for better growth and maximum reproduction.

Peak cocoon production was observed on the 6th week in control and in the 7th week in T1 and T3. It was followed by a gradual decline in control, T1 and T3. In T2 cocoon production and earthworm survival showed a declined trend due to the acidic condition of crude spent wash. There was not much variation in the production of cocoons unlike the findings of Jawria et al, where there was an increase in the production of cocoons till the end of the experiment with the same substrate material.

The cocoon produced immediately seems to be soft and light cream in colour similar to that observed by Reinecke and Viijoen (1988) which slowly harden and changed to yellow brown. The hatching viability was highest in T3 (93.05 ± 0.14) followed by T1 and control. The significant*(P < 0.01)*positive correlation between number of hatchlings per cocoon ,high rate of cocoon production, short development time with high hatching success, indicate that *E. Eugeniae* can be a candidate species in vermiculture with distillery spent wash as a major component.

Conclusion

The feasibility of using earthworms for waste management as well as a potential source for protein in animal nutrition depends on a fundamental knowledge of the basic parameters governing the survival, growth and reproduction of earthworm species. The present study emphasized that the reproductive potential of earthworms is highly influenced by the quality and availability of food (Hussain *et al* 2016. It was concluded that the obtained distillery spent wash when diluted with water at 1:3 ratio and after partial decomposition of waste material, works as an excellent pallatable raw material for vermicomposting using *Eudrilus eugeniae* earth worm. It showed that the earthworms were showing a similar trend in growth, reproduction, fecundity, hatching viability and that when reared in conventional vermibed comprising of domestic waste and cattle dung. The short incubation period, rapid growth rate and size could, however, favour *E. eugeniae* as a commercial breeder and as a potential protein source. The investigation have established the viability of using earthworm, *Eudrilus eugeniae* as a decomposer for degrading spent wash and converting it into a value added biofertilizer and the cultured earthworm as a protein source for brood stocks in aquariculture.

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