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ORIGINAL ARTICLE

Growth and reproduction performances of earthworm (*Perionyx excavatus*) fed with different organic waste materials

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ABSTRACT

Objective: The purpose of the experiment was to examine the effect of different food sources on the growth and reproduction performances of an epigeic earthworm *Perionyx excavatus*.
Materials and Methods: The experiment was carried out in 18 cylindrical plastic containers for 10 weeks. The study was designed with six treatments, each having three replicates. In control treatment (T_1), cow dung was used as the only food source for the earthworm. In another five treatments, water hyacinth (T_2), chopped banana plant trunk (T_3), vegetable scrap (T_4), paddy straw (T_5), and sugarcane bagasse (T_6) were used as food sources with cow dung as bedding material.
Results: The maximum weight gain of earthworm *P. excavatus* was 3,294.7 ± 4.5 mg for the food staff of vegetable scrap (T_4). Earthworm *P. excavatus* fed with chopped banana plant trunk (T_3) showed a very similar weight gain of 3,243.7 ± 3.8 mg. On the contrary, the minimum weight gain was 1,799.7 ± 3.5 mg for the food staff of paddy straw (T_5). The maximum cocoon number of 137.33 ± 6.46 mg was observed in T_3, whereas a minimum number of 36.67 ± 4.16 mg in T_1. The highest number of hatchlings (12.33 ± 0.88 mg) was recorded in treatment T_3, whereas the least number of hatchling (5.00 ± 0.58 mg) was observed in T_1.
Conclusion: It was concluded that, among the six different food sources, chopped banana plant trunk was preferable food source to the earthworm for growth and reproduction.

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Perionyx excavatus; organic food sources; weight gain; cocoon; hatching success.

Introduction

Earthworms are important soil invertebrate animals found in many parts of the world both in the temperate and tropical regions [1]. Among them, earthworm *Perionyx excavatus* is an epigeic earthworm species requiring an adequate amount of suitable organic matter and high moisture contents for proper growth and reproduction [2,3]. The earthworm (*P. excavatus*) is used in vermicompost preparation [4,5], organic agriculture [6], and as feed for chicken, pig, and fish species [7–9]. The earthworm is an excellent source of high-quality animal protein, essential amino acids, and other nutrients including fats, vitamins, and minerals [10]. Arancon et al. [11] found 60%–70% of protein, 7%–10% of fat, 8%–20% of carbohydrate, and 2%–3% of minerals in earthworms in dry matter basis, but the supply of earthworm from natural habitat cannot meet the global demand as an animal feed supplement. Moreover, the abundance and distribution of earthworms in natural habitat are seasonal [12,13]. Earthworm also has a high tendency to bioaccumulate the toxic organic residues including pesticides, herbicides, antibiotics, and heavy metals into their tissue [1,14]. Therefore, there is a risk of transferring those toxicants in poultry, fishes, and ultimately humans through a wild earthworm if used [1]. Therefore, earthworm rearing has recently been very popular to supply earthworm around the year and to avoid the biomagnifications of these hazardous pollutants. Earthworm production can help in the process of waste degradation, and the produced earthworm could be used as an alternate animal protein source for livestock, poultry, and fish.

The life cycle of earthworm *P. excavatus*, its reproductive biology, its efficacy in waste decomposition, and its rearing

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using organic wastes are substantially reported [4,5,15,16]. However, the impact of food sources on the growth and reproduction of this species is still scarcely studied. Hence, the experiment was conducted to identify the effect of different food sources on the growth and reproduction performance of earthworm *P. excavatus*. This is because the food sources used in this experiment were cheap and easily available, and those food materials are mostly organic waste materials; disposal of those large quantities of wastes can cause serious environmental problems if not properly managed.

Materials and Methods

Preparation of bedding and feeding material

In this experiment, cow dung was used as bedding material. Five types of feeding materials, namely, water hyacinth, chopped banana plant trunk, vegetable scrap, paddy straw, and sugarcane bagasse were used in treatment T_2–T_6, respectively. In treatment T_1 (control), cow dung weighing 4,000 gm was used as bedding material, and in other treatments, the bedding and feeding materials were given into 3:1 ratios. All the bedding and feeding materials were chopped into small pieces and dried for 48 h because earthworms do not survive in fresh cow dung and organic wastes. Then, all they were kept for prior microbial composting and thermo-stabilization for 15 days. The moisture content of 70%–80% was retained by sprinkling water daily in the beddings.

Culture of P. excavatus

The experiment was conducted in the Wet Laboratory of the Department of Aquaculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh. The experiment was laid out in a completely randomized design. There were six treatments (T_1, T_2, T_3, T_4, T_5, and T_6) each having three replications. The earthworms were collected from a commercial earthworm farm situated in Pubail, Gazipur, Bangladesh. A total of 18 cylindrical plastic containers (a diameter of 14 cm and a depth of 12 cm) were used in this experiment. In each container, 10 young non-clitellated earthworm *P. excavatus* were inoculated. At first, they were collected from the stock culture, cleaned with distilled water, weighed with electronic balance, and then finally inoculated into each container. The earthworms were reared for 10 weeks. Additional bedding or feeding materials were never added during the experimental period.

Sampling

Sampling was conducted weekly to observe the growth and maturation of earthworms. The soil quality parameters such as soil temperature (25.1°C–28.5°C), soil pH (6.5–7.5), and soil moisture (75.5%–82.7%) recorded during the experimental period were found to be within the optimum range for earthworm culture. Soil temperature was recorded by using the Celsius thermometer (digi- thermo WT-2). Soil pH and moisture were recorded by using a soil tester (DM-15).

Determination of growth performance

The weight of earthworms was measured weekly in each container to decide the growth performance. Earthworms were separated from the substrate manually, cleaned in tap water to remove adhering substrate materials from the body, and then weighed on a live weight basis. Finally, all the earthworms were returned to their respective containers. Based on the obtained data, growth parameters such as weight gain and growth rate (mg/worm/day) were calculated.

Determination of reproduction performance

A substrate from each container was checked weekly to observe the onset of cocoon production. When cocoons appeared, they were separated manually and washed carefully in distilled water, and the total number of cocoons was recorded to figure out the fecundity. To determine the hatching success, 10 freshly laid cocoons from each container were transferred to small plastic boxes (11 cm × 5 cm × 5 cm) containing the same bedding material, in which their parents were reared. The boxes were checked daily to monitor the emergence of hatchlings. Immediately after the hatchling appears, they were separated manually by using a fine painting brush and counted to determine the total number of hatchlings that emerged.

Determination of the chemical composition of end soil

At the end of the experiment, the soil was analyzed in Bangladesh Rural Advancement Committee (BRAC) Agricultural Research Center, Gazipur, Bangladesh. Nitrogen was determined by using a Kjeldahl apparatus (TDK 152) following the micro-Kjeldahl method. Phosphorous was determined by using an autoanalyzer (Tecator, model 5012). Exchangeable potassium was determined with an atomic absorption spectrophotometer (Perkin Elmer, 3110). Soil organic matter was analyzed by Walkley–Black method [5].

Statistical analysis

The data were analyzed with a one-way analysis of variance to find out whether there were any significant differences among treatment means, whereas the Least Significant Difference (LSD) test was used to compare the treatment means using SPSS v 16.

Results and Discussion

Growth performance of P. excavatus

The growth pattern of earthworm *P. excavatus* over the experimental period of 10 weeks is shown in Figure 1. The weight of the earthworms in all the treatments increased gradually with age from week 1 onward. The earthworm attained the maximum weight in the 5th week in control (T_1), chopped banana plant trunk (T_3), and vegetable scrap (T_4) treatments, whereas it attained the maximum weight at 4th week in water hyacinth (T_2), paddy straw (T_5), and sugarcane bagasse (T_6) treatments. The maximum earthworm weights of 5,733.7 ± 5.0 and 5,356.0 ± 3.5 mg were observed in vegetable scrap (T_4) and chopped banana plant trunk (T_3), respectively, whereas the significantly lower weight of 3,645.7 ± 5.5 mg was observed in paddy straw (T_5). The weight was followed by weight loss by the time of completion of the study. The weight loss might be associated with the depletion of food [17,18]. Jesikha and Lekeshmanaswamy [19] reported a related pattern of weight loss when earthworms attained sexual maturity because earthworm utilizes the energy for reproduction purposes such as copulation, cocoon formation, and egg laying.

In this study, a maximum weight gain of 3,294.7 ± 4.5 mg was attained in vegetable scrap, followed by chopped banana plant trunk (3,243.7 ± 3.8 mg), and minimum weight gain of 1,799.7 ± 3.5 mg was obtained in paddy straw. The growth rate (mg/worm/day) of earthworm *P. excavatus* in T_1, T_2, T_3, T_4, T_5, and T_6 treatments was 3.11, 2.83, 4.63, 4.71, 2.57, and 3.59 mg/earthworm/day, respectively (Table 1). According to Meharaj and Manivannan [20], the growth rate (mg/earthworm/day) is an excellent and acceptable comparative index to compare the growth of earthworms in different organic wastes. In this study, earthworm, *P. excavatus*, which were fed vegetable scrap and chopped banana plant trunk, showed better growth rates (4.71 ± 0.01 and 4.63 ± 0.01 mg/earthworm/day) respectively, whereas paddy straw-fed *P. excavatus* showed the least growth rate of 2.57 ± 0.01 mg/earthworm/day. The growth rate of the earthworm is affected by the type and quality of feeding material [21]. However, growth rate

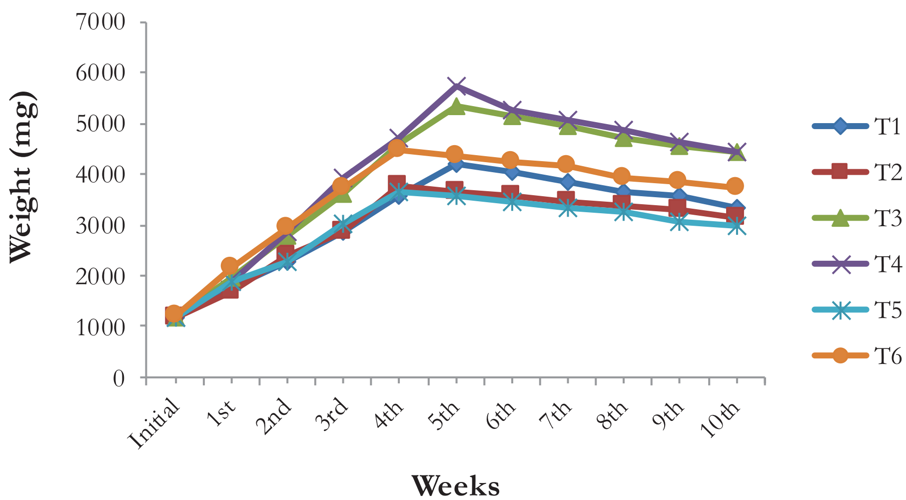

Figure 1. Growth pattern of earthworm *P. excavatus* over the experimental period of 10 weeks [T_1 = control (only cow dung), T_2 = cow dung + water hyacinth (3:1), T_3 = cow dung + chopped banana tree trunk (3:1), T_4 = cow dung + vegetable scrap (3:1), T_5 = cow dung + paddy straw (3: 1), and T_6 = cow dung + sugarcane bagasse (3:1)].

Table 1. Growth performance of earthworm *P. excavatus* in different treatments.

Treatment	Average initial weight (mg)	Maximum weight achieved (mg)	Maximum weight found on (mg)	Average final weight (mg)	Weight gain (mg)	Growth rate (mg/worm/day)
T_1	1,165.0 ± 2.2	4,209.3 ± 6.6	5th week	$3,343^c$ ± 2.6	$2,178.0^c$ ± 2.5	3.11 ± 0.00
T_2	1,163.0 ± 2.5	3,777.3 ± 4.5	4th week	$3,146^d$ ± 3.6	$1,983.0^d$ ± 4.4	2.83 ± 0.01
T_3	1,187.3 ± 3.9	5,356.0 ± 3.5	5th week	$4,431^a$ ± 1.1	$3,243.7^a$ ± 3.8	4.63 ± 0.01
T_4	1,163.3 ± 3.4	5,733.7 ± 5.0	5th week	$4,458^a$ ± 3.8	$3,294.7^a$ ± 4.5	4.71 ± 0.01
T_5	1,185.3 ± 2.4	3,645.7 ± 5.5	4th week	$2,985^e$ ± 2.0	$1,799.7^e$ ± 3.5	2.57 ± 0.01
T_6	1,207.0 ± 2.8	4,499.3 ± 4.3	4th week	$3,733^b$ ± 4.4	$2,526.0^b$ ± 2.3	3.60 ± 0.00

T_1 = control (only cow dung), T_2 = cow dung + water hyacinth (3:1), T_3 = cow dung + chopped banana tree trunk (3:1), T_4 = cow dung + vegetable scrap (3:1), T_5 = cow dung + paddy straw (3:1), T_6 = cow dung + sugarcane bagasse (3:1) [Values bearing different superscript are significantly different ($p < 0.05$)].

recorded in this experiment was more or less similar to that reported by Birundha et al. [22], where earthworm *P. excavatus* was examined in different biodegradable organic wastes and gained highest growth rate of 4.81 ± 0.47 and 4.30 ± 0.38 mg/earthworm/day in pressmud and leaf litter, respectively. In this study, the growth performances of *P. excavatus* were better in vegetable scrap (T_4) and chopped banana tree trunk (T_3) treatments.

Reproductive performance of P. excavatus

Cocoon production

The development of clitellum and cocoon production by earthworm *P. excavatus* in different treatments is shown in Table 2. Clitellum development in earthworm individuals in all the treatments was observed from the 2nd week of the experiment, and cocoon production was started from the 3rd week of the experiment. In the previous studies, mean clitellum development period was 3–4 weeks and cocoon production started between 4 and 5 weeks in different organic wastes [22,23]. In the present experiment, clitellum development in earthworms occurred earlier than the previous study, and it may be due to the inoculation of young non-clitellated earthworms in culture media. The cocoon production ceased after 7th week in T_1, T_2, T_3, T_4, and T_5 treatments, whereas after 8th week in T_6 treatment (Fig. 2). After 10 weeks, maximum cocoons (137.33 ± 6.46) were obtained in T_3 (chopped banana plant trunk) and minimum (36.67 ± 4.16) in T_1 (control) treatment. The variation in the number of cocoons produced could be due to the influence of feeding materials and the nutritional value of the culture medium [24,25]. The presence of a high level of total potassium and total calcium in the chopped banana plant trunk may be the factors behind the better reproductive performance of earthworm *P. excavatus*. The highest cocoon production rate (0.39 ± 0.02 cocoon/worm/day) was obtained in T_3, whereas the lowest (0.10 ± 0.02 cocoon/worm/day) in T_1. Therefore, the

Table 2. Cocoon production by earthworm *P. excavatus* in different treatments.

Treatment	Clitellum development started in	Cocoon production started in	No. of cocoon produced in 10 weeks	No. of cocoons produced/worm	No. of cocoon produced/worm/day	Cocoon production ceased after
T_1	2nd week	3rd week	$36.67^{d} \pm 4.16$	3.67 ± 0.42	0.10 ± 0.02	7th week
T_2	2nd week	3rd week	$70.67^{c} \pm 6.27$	7.07 ± 0.63	0.20 ± 0.02	7th week
T_3	2nd week	3rd week	$137.33^{a} \pm 6.46$	13.73 ± 0.65	0.39 ± 0.02	7th week
T_4	2nd week	3rd week	$98.33^{b} \pm 6.41$	9.83 ± 0.64	0.28 ± 0.02	7th week
T_5	2nd week	3rd week	$80.67^{bc} \pm 6.02$	8.07 ± 0.60	0.23 ± 0.02	7th week
T_6	2nd week	3rd week	$134.00^{a} \pm 5.71$	13.4 ± 0.57	0.31 ± 0.01	8th week

T_1 = control (only cow dung), T_2 = cow dung + water hyacinth (3:1), T_3 = cow dung + chopped banana tree trunk (3:1), T_4 = cow dung + vegetable scrap (3:1), T_5 = cow dung + paddy straw (3:1), T_6 = cow dung + sugarcane bagasse (3:1) [Values bearing different superscripts are significantly different ($p < 0.05$)]

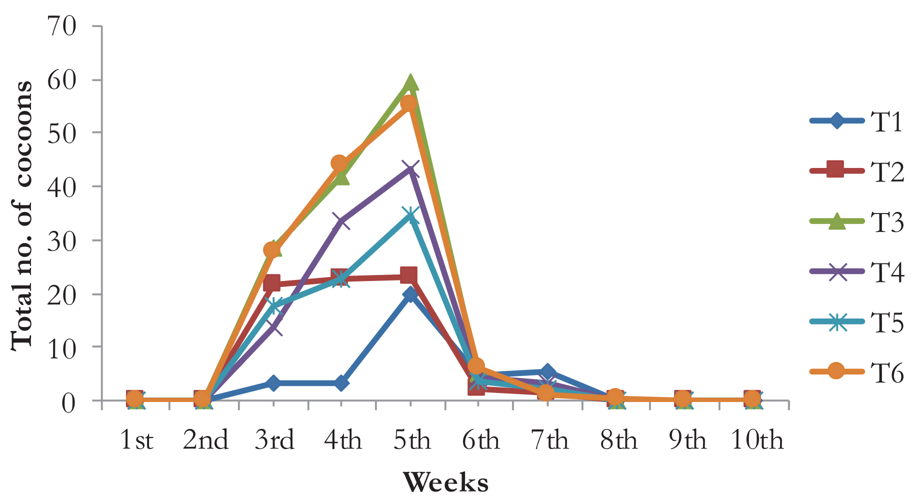

Figure 2. Weekly production of cocoon by earthworm *P. excavatus* in different treatments [T_1 = control (only cow dung), T_2 = cow dung + water hyacinth (3:1), T_3 = cow dung + chopped banana tree trunk (3:1), T_4 = cow dung + vegetable scrap (3:1), T_5 = cow dung + paddy straw (3:1), and T_6 = cow dung + sugarcane bagasse (3:1)].

reproduction rate was found significantly higher in T_3, and according to Chauhan and Singh [26], it may be due to good aeration in chopped banana plant trunk. Suthar [27] also reported a similar cocoon production rate of 0.15–0.23 cocoons/worm/day on different organic wastes.

In the present study, the highly significant ($p < 0.05$) cocoon production was observed in treatment T_3, where *P. excavatus* was fed with chopped banana plant trunk. Chopped banana plant trunk requires less time to decompose and has a sufficient amount of non-assimilated carbohydrates which increased the cocoon production performance [28]. Similarly, Nath and Chaudhuri [29] also reported that food sources with a significant amount of easily metabolizable organic matter facilitate growth and reproduction in the earthworm. The weight gain by earthworm was higher in vegetable scrap (T_4), but cocoon production was lower in this treatment than chopped banana plant trunk (T_3). It indicates that vegetable scrap (T_4) is not suitable for reproduction but suitable for a biomass supporting medium.

Hatching success of cocoons

A significant variation in hatching success of cocoons of earthworm *P. excavatus* was noticed in different treatments, which is shown in Table 3. The maximum number of hatchlings (12.33 ± 0.88) was recorded in the chopped banana plant trunk (T_3), whereas the least hatchling number (5.00 ± 0.58) was observed in control (T_1). The number of hatchlings emerged in treatment T_3 was found to be significantly higher ($p < 0.05$) than the other five treatments. The highest hatching percentage (99.97%) was recorded in chopped banana plant trunk (T_3), whereas the lowest (50.13%) in control (T_1). Birundha et al. [22] reported that the cocoons of earthworm *P. excavatus* that developed in different organic wastes had a hatching success of 48.05%–67.24%. However, in this experiment, the best hatching success was recorded in treatment T_3, which showed a better result than the previous studies. There are limited published reports about the influence of substrate quality on cocoon hatching success, but it is, perhaps, due to the presence of a high level of nitrogen content in the substrate. Degefe and Tamire [25] assumed that nutrients, especially N-content in the substrate, has a strong influence on hatching success of earthworm *P. excavatus.*

Characteristics of the end soil

The soil of different treatments was examined for organic matter, total N, total phosphorus, and potassium at the termination of the study (Table 4). Here, the amount of organic matter, N (%), P (%), and K (%) is higher in the soil of T_3, where chopped banana tree trunk was used as a feeding material than all other treatments. Soil rich in organic matter and N content affects the growth and reproduction of earthworm [18,30]. Therefore, T_3 treatment provides

Table 3. Hatching performance of cocoons of *P. excavatus* in different treatments.

Treatment	Total no. of hatched cocoons	Hatching success (%)	No. of hatchling emerged	Hatchling/cocoon
T_1	$5.05^c \pm 0.17$	$50.13^c \pm 0.12$	$5.00^c \pm 0.58$	0.50
T_2	$8.17^b \pm 0.15$	$80.54^b \pm 0.61$	$9.67^b \pm 0.33$	0.97
T_3	$9.98^a \pm 0.21$	$99.97^a \pm 0.35$	$12.33^a \pm 0.88$	1.23
T_4	$6.34^c \pm 0.13$	$60.34^c \pm 0.27$	$6.67^c \pm 0.88$	0.67
T_5	$7.12^c \pm 0.11$	$70.87^c \pm 0.19$	$7.00^c \pm 0.58$	0.70
T_6	$8.17^b \pm 0.15$	$80.54^b \pm 0.61$	$9.67^b \pm 0.88$	0.97

T_1 = control (only cow dung), T_2 = cow dung + water hyacinth (3:1), T_3 = cow dung + chopped banana tree trunk (3:1), T_4 = cow dung + vegetable scrap (3:1), T_5 = cow dung + paddy straw (3:1), T_6 = cow dung + sugarcane bagasse (3:1) [Values bearing different superscripts are significantly different ($p < 0.05$)].

Table 4. Chemical properties of end soil of different treatments at the end of the experiment.

Treatment	Organic matter (OM) %	Nitrogen (N) %	Phosphorus (P) %	Potassium (K) %
T_1	13.42	1.56	1.14	0.44
T_2	16.10	1.44	1.01	0.44
T_3	17.44	1.73	1.11	0.87
T_4	10.73	1.27	0.87	0.63
T_5	16.10	1.41	0.77	0.52
T_6	8.05	1.53	1.00	0.56

T_1 = control (only cow dung), T_2 = cow dung + water hyacinth (3:1), T_3 = cow dung + chopped banana tree trunk (3:1), T_4 = cow dung + vegetable scrap (3:1), T_5 = cow dung + paddy straw (3:1), T_6 = cow dung + sugarcane bagasse (3:1).

a suitable condition for better growth and reproduction. Moreover, this soil can be used as a fertilizer in agriculture land as it has a higher amount of nitrogen, phosphorus, and potassium. Chauhan and Sing [31] reported significantly the highest total potassium and total calcium in banana pills, and the amount was 8.3 ± 0.36 gm/kg and 3.8 ± 0.42 gm/kg, respectively.

Conclusion

The use of appropriate food sources is important for earthworm *P. excavatus* culture to increase the growth and reproduction performance. The weight of earthworm *P. excavatus* was achieved better when vegetable scrap was used as a food source followed by a chopped banana plant trunk. A higher reproductive performance of earthworm *P. excavatus* was recorded in chopped banana plant trunk treatment. The quality of vermicompost was also improved by feeding chopped banana plant trunk to *P. excavatus*. Therefore, among the five different food sources, chopped banana plant trunk was preferable as feed for culture and higher reproductive performance of earthworm *P. excavatus*.

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Conflict of interest

There is no conflict of interest

Authors' contribution

Masuma Akter Sadia, Md. Amzad Hossain, and Md. Rabiul Islam designed the study. Md. Amzad Hossain, Md. Rabiul Islam, and Dinesh Chandra Shaha supervised throughout the experimental work while Masuma Akter Sadia conducted the work. Taslima Akter assists are designing an implementation of the study. All authors contributed to write and review the manuscript and approved the final manuscript.

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