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Research Article

Life Cycle Patterns of Epigeic Earthworm Species (*Eisenia fetida*, *Eisenia andrei*, and *Dendrobaena veneta*) in a Blend of Brewery Sludge and Cow Dung

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Comprehending the growth and reproductive capabilities of vermicomposting worm species across diverse wastes from various sources is crucial for the effective utilization of earthworms in sustainable waste management systems. This study evaluated the growth and reproductive potential of three epigeic earthworm species in a substrate comprising brewery sludge mixed with cow dung. Following the introduction of sexually matured earthworm species into the experimental substrate, *E. fetida* released 165 ± 0.88 cocoons at a reproduction rate of 32.8 ± 0.67 cocoons/worm, *E. andrei* released 164 ± 1.48 cocoons at a cocoon production rate of 32.19 ± 1.38 cocoons/worm, and *D. veneta* released 110.25 ± 1.2 cocoons at a cocoon production rate of 22.7 ± 1.5 cocoons/worm. The incubation period for *E. fetida* and *E. andrei* ranged between 3 and 4 weeks, while *D. veneta* required a relatively longer period of 5 to 7 weeks. After the introduction of newly hatched hatchlings into the test substrate, a similar pattern of biomass increment was observed between *E. fetida* and *E. andrei*, with both species exhibiting progressive growth up to the 5th week. In *D. veneta*, successive biomass increments were recorded up to the 7th week, after which the biomass of each experimental species gradually declined. The current study demonstrated that all experimental earthworm species exhibited robust growth and reproductive performance in brewery waste mixed with cow dung.

1. Introduction

Presently, the agro-based industrial sectors, particularly the brewery industries in Ethiopia, are experiencing significant growth. However, the generated waste from these industries poses substantial environmental challenges [1]. As per the findings of Oljira et al. [2], these industries in Ethiopia often release a massive amount of waste with minimal or no treatment. Consequently, beverage industries, and more specifically brewery industries, are recognized for their environmental impact due to the discharge of untreated or inadequately treated effluents and various forms of waste into nearby water bodies and soil matrices. In general, the brewery industry is characterized by the production of substantial amounts of diverse wastes, including hot trub, spent grain, yeast cells, kieselguhr, and brewery sludge [3]. Among these, brewery sludge stands out as the most challenging waste to manage due to its varied and fluctuating composition [4, 5]. Moreover, the disposal of brewery sludge has proven to be costly [6]. These wastes contain various types of hazardous organic pollutants that could adversely affect the environment [1].

Brewery sludge, being organic in nature, provides an opportunity to adopt ecofriendly, cost-effective, and innovative biological management methods such as vermicomposting rather than dumping to disposal in landfill sites. Vermicomposting is a straightforward, nonthermophilic ecobiotechnological technique that transforms complex organic substances into stabilized, nutrient-rich, and microbiologically active soil amendments under aerobic conditions [7–10]. The primary objective of vermicomposting is to manage organic wastes in an environmentally friendly manner and produce vermicompost [11]. This practice is typically carried out through the collaborative efforts of specific earthworm species and microorganisms [12].

Vermicomposting is regarded as an alternative and innovative technology that is environmentally friendly, cost-effective, and relatively newfangled [13]. Consequently, it has become one of the fastest-growing sectors in environmental management. During the vermicomposting process, various ecological, physical, and biochemical transformations occur through the combined action of earthworms and microorganisms [14]. The biochemical reactions are primarily facilitated by microorganisms, while earthworms play a crucial role in driving the entire process. Physically, they blend, fragment, and aerate the substrate, significantly altering the waste [15] and enhancing microbial activity. Their actions also increase the surface area for microbial activity, further stimulating decomposition [12, 16].

Earthworms avidly consume organic wastes, utilizing only a small portion for their bodily synthesis and excreting a significant portion in a semidigested form known as vermicast. Vermicast constitutes the major portion of the end product of the vermicomposting process [9] and contains a wide range of microflora that enhances further decomposition, along with enzymes and hormones [17]. Understanding the life cycle patterns of vermicomposting worms in various industrial, municipal, or household wastes is crucial for the effective application of these earthworm species in a sustainable waste management system [14, 18]. The prolific decomposition potential of earthworms, as more population can decompose vast amounts of wastes [19], makes the growth and reproductive performance of different earthworm species in various substrates valuable biomarkers to measure the suitability of earthworm species for vermicomposting [20, 21]. Despite numerous studies on the life cycle performance of common vermicomposting earthworm species on a variety of wastes from industrial and nonindustrial sources, little to no research has been conducted on the growth and reproductive potential of various earthworm species on brewery sludge. Recognizing this gap, this study was undertaken to assess the suitability of brewery sludge for the survival, growth, and reproduction of three vermicomposting epigeic earthworm species: *E. fetida*, *E. andrei*, and *D. veneta*.

2. Materials and Methods

In this life cycle experiment, the growth and reproductive potential of three epigeic earthworm species, namely, *E. fetida*, *E. andrei*, and *D. veneta*, were assessed in brewery factory sludge mixed with cow dung. The experiment was conducted in small plastic containers measuring 12.7 cm in depth and 10.16 cm in diameter from March 2022 to July 2022. The experiments took place in an earthworm farm located in Kotu town, North Shewa Zone, Ethiopia, and the limnology laboratory of Addis Ababa University. To create a suitable substrate for the worms, the sludge was mixed with powdered cattle dung in a 3 : 1 ratio. Various life cycle patterns, including cocoon production rate, cocoon incubation period, hatching success (number of hatchlings), biomass, and maturation, were studied. The experiments were conducted at an average room temperature of 27°C. The moisture level of the sample substrates was maintained between 60 and 70% throughout the study period. Moisture determination involved taking samples of the substrate at 3-day intervals, weighing them, and drying them in an oven at 105°C for 24 hours. The oven-dried samples were then cooled for an hour in a desiccator and reweighed. The percentage moisture content was computed using the equation MAFF [22], and the variation between moist and dried samples was calculated. The moisture content of the beddings was adjusted accordingly based on this variation [23].

2.1. Experimental Design for Fecundity Assessment (Cocoon Production Rate). The study utilized nine cylindrical plastic containers, with three replicates for each combination of worm-brewery sludge mixed with cow dung. Each container was adequately filled with the experimental substrate to ensure a continuous food supply during the reproductive period. Ten newly clitellated individuals of *E. andrei*, *E. fetida*, and *D. veneta* were sourced from an earthworm farm in Deneba and Kotu town, North Shewa Zone, Ethiopia. Before introduction into the containers containing brewery sludge mixed with cow dung, the worms underwent a bath with distilled water to remove any adhering substances. Following a brief drying on paper towels, the worms were weighed using an electronic balance. Daily observations were conducted for each experimental treatment to record the initiation of cocoon production. Upon cocoon observation, they were hand-sorted, briefly rinsed in distilled water, and counted to determine the fecundity (cocoon/worm/day) [24].

2.2. Experimental Approach for Determining Cocoon Incubation Period, Hatching Success, and Hatchling Count. To assess the incubation period (the duration from cocoon production to the emergence of the first hatchling), hatching success (total number of hatched cocoons), and the number of hatchlings per cocoon, fifteen recently laid cocoons were selected from the containers used for fecundity determination. These cocoons were then introduced into containers containing the same substrate in which their parent worms were raised. Each worm species, along with the specific experimental waste combination, was arranged in triplicate. For determining the incubation period, daily observations were conducted on the treatments to monitor the appearance of hatchlings. Once hatching commenced, the hatchlings were systematically removed on a daily basis using a paintbrush. Subsequently, the hatchlings were manually sorted and counted to calculate the total number of neonates or hatchlings originating from a single cocoon. Simultaneously, the number of nonhatched cocoons was tallied in each experimental container to establish the hatching success of cocoons for each worm species included in the study [25].

2.3. Experimental Design for Biomass and Maturation Assessment. For the investigation of changes in biomass and sexual maturation, the same type of test substrate and containers used for determining reproductive performance were employed. Each experimental container was adequately filled with the experimental diet to ensure a consistent food supply. In each replicate, ten freshly emerged hatchlings of each experimental worm species were selected from the containers used for determining the cocoon incubation period. Before introducing the hatchlings into the respective containers with the experimental substrate (brewery sludge mixed with cow dung), they were cleaned with distilled water to remove any adhering substances, briefly dried on paper towels, and weighed using an electronic balance. To track growth and biomass progression, the hatchlings in each treatment were weighed weekly for fifteen consecutive weeks. Three life cycle stages were identified: preclitellate, distinguished by the occurrence of tubercula pubertasis; clitellate, characterized by a well-developed clitellum; and the loss of the clitellum or regression. The date of the appearance of the succeeding generation of cocoons was also recorded. Based on the obtained data on worm biomass, the following parameters were calculated: the percentage ratio of the maximum weight attained to the initial body weight or relative growth rate (g/earthworm/day), maximum weight gained, net weight achieved, and the percentage weight gained over time (specific growth rate) [25].

2.4. Statistical Analysis. To discern statistical differences in cocoon production, hatchling success, and the quantity of hatchlings/neonates per cocoon among the three worm species, the data underwent one-way analysis of variance (ANOVA) at a significance level of $P < 0.05$, utilizing the SPSS 15.0 software package. The weight obtained was calculated based on the difference between the initial biomass of the introduced earthworms and the maximum biomass achieved.

3. Results

3.1. Cocoon Production Performance. Following the introduction of sexually matured earthworm species into the experimental substrate, the cocoon production performance was observed. *E. fetida* exhibited a cocoon release of 165 ± 0.88, with a reproduction rate of 32.8 ± 0.67 cocoons per worm. *E. andrei* released 164 ± 1.48 cocoons, resulting in a cocoon production rate of 32.19 ± 1.38 cocoons per worm. On the other hand, *D. veneta* released 110.25 ± 1.2 cocoons, with a cocoon production rate of 22.7 ± 1.5 cocoons per worm (Table 1). For *E. fetida* and *E. andrei*, cocoon production consistently increased from the 4th week up to the 7th week. In contrast, *D. veneta* demonstrated a successive rise in cocoon production from the 7th week up to the 11th week. Subsequently, the cocoon production of all three worm species progressively declined and eventually ceased (Figure 1). No significant difference was observed between the cocoon production performance of *E. fetida* and *E. andrei* in brewery sludge mixed with cow dung ($P > 0.05$). However, a significant variation was noted between the cocoon production potential of *D. veneta* and *E. fetida*, as well as *D. veneta* and *E. andrei*, in the experimental diet of this study ($P < 0.05$).

3.2. Incubation period, Hatching Success, and Neonates per Cocoon. The incubation period for both *E. fetida* and *E. andrei* ranged from 3 to 4 weeks, while *D. veneta* took a relatively longer period, spanning between 5 and 7 weeks. In terms of the total number of neonates or hatchlings, *E. fetida* recorded 52 ± 0.88, with an average of 3.5 hatchlings per cocoon. *E. andrei* had a total of 50 ± 0.7 neonates, averaging 3.3 hatchlings per cocoon. On the other hand, *D. veneta* had the highest number of neonates, with 14.33 ± 0.33 and an average of 0.95 neonates per cocoon. Unhatched cocoons were minimal across all tested earthworm species. Upon emergence, the worms appeared white without pigmentation, gradually developing their distinctive adult coloration over a short period. The performance of hatching was noteworthy for all experimental species (Table 2).

3.3. Growth, Sexual Maturation, and Cocoon Initiation. Following the introduction of newly hatched hatchlings into the test substrate, a similar pattern of biomass increment was observed between *E. fetida* and *E. andrei*, with both species showing progressive growth up to the 5th week. In *D. veneta*, successive biomass increment as recorded up to the 7th week. However, after these weeks, the biomass of each experimental species gradually declined (Figure 1). Regarding sexual maturity, the worms were considered sexually mature when they displayed a well-developed and swollen clitellum [12]. In this study, upon the introduction of hatchlings into the test substrate, the first preclitellate worms (identified by the occurrence of tubercula pubertasis) were observed in the 3rd week, and the first sexually mature individuals with well-developed clitellum were observed in the 4th week in *E. fetida* and *E. andrei*. Both species commenced cocoon release on the 4th week. The minimum maturation time from the appearance of the hatchling from the cocoon to complete clitellum development was four weeks in these two species. Moreover, the average time to complete the life cycle (from a freshly laid cocoon, through the incubation period, over the clitellate worm, to the appearance of the subsequent generation of cocoon) was in the range of seven to eight weeks.

In the experimental treatment of this study, *D. veneta* exhibited preclitellate characteristics by the 5th week, with mature individuals featuring fully developed clitella observed by the 7th week. The first cocoon appeared on the 7th week. The minimum duration for maturation, from the emergence of neonates from the cocoon to full clitellum development, was about seven weeks. The overall life cycle took an average of 10 to 14 weeks (5–7 weeks of incubation period and 7 weeks until the first cocoon was released) (Table 3).

Table 1: Cocoon production by experimental earthworm species after introducing into experimental treatments.

Reproductive parameters	Experimental earthworm species		
	E. fetida	*E. andrei*	*D. veneta*
Minimum developmental time to start cocoon production	4 week	4 week	7^{th} week
Mean number of cocoon produced	165 ± 0.88	164 ± 1.48	110.25 ± 1.2
Cocoon production/worm	32.8 ± 0.67	32.19 ± 1.38	22.7 ± 1.35
Mean cocoon production/week (in batch)	18.2 ± 1.97	18.33 ± 1.73	10.02 ± 1.4
Mean cocoon production/worm/week	3.6 ± 0.87	3.6 ± 1.76	2 ± 1.97
Mean cocoon production/worm/day	0.52 ± 0.83	0.52 ± 0.91	0.29 ± 1.2
Time to cease cocoon production	11^{th} week	11^{th} week	13^{th} week

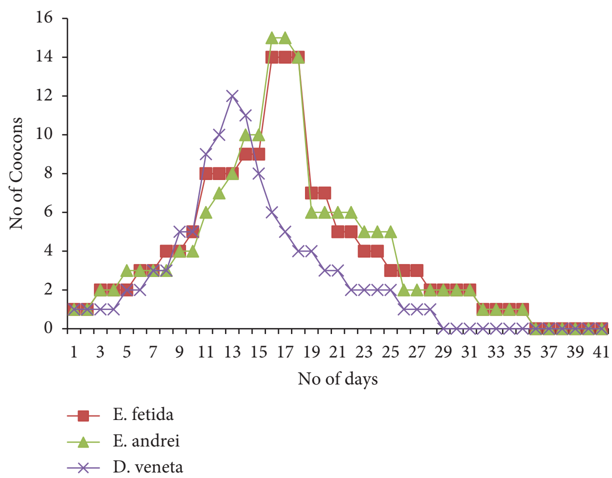

Figure 1: Cocoon production pattern.

Table 2: Incubation period and hatching performance of cocoons of *E. fetida*, *E. andrei*, and *D. veneta*.

	Earthworm species		
	E. fetida	*E. andrei*	*D. veneta*
Incubation period (in days)	21–26	21–27	35–44
Total number of hatched cocoons	13 ± 0.05	13 ± 0.03	12 ± 0.03
Hatching success	86.6%	86.6%	80%
Total number of hatchlings emerged	52 ± 0.88	50 ± 0.66	14.33 ± 0.33
Hatchlings/cocoon	3.5	3.3	0.95

4. Discussion

Understanding the reproductive and growth behavior of vermicomposting worms on different types of wastes is crucial for successful vermicomposting and vermiculture [14, 18, 26]. Numerous studies confirm that, in addition to inherent characteristics, the growth and reproductive performance of vermicomposting earthworm species depend on the physicochemical properties, palatability, and microbial composition of their feeding substrate [20, 24, 25, 27]. The data obtained from the current trial also demonstrates that the quality of the feed substrate used for vermicomposting can influence the reproductive performance and biomass production of vermicomposting earthworms. The average cocoon production rate of *E. fetida* in the experimental substrate of this study aligns with the reported average cocoon production rate (0.5 cocoons/worm/day) by Venter and Reinecke [28]. However, the cocoon production rate of *D. veneta* in this treatment was notably lower compared to that reported by Fayolle et al. [29] (0.74 cocoons/worm/day).

In general, all species of worms in this experiment exhibited substantial biomass gain. This biomass gain could be attributed to the biochemical nature of the feed substrate, a crucial factor in determining the growth and reproductive performance of vermicomposting worms [30]. Brewery sludge, being rich in nitrogen content, easily decomposes, and when mixed with cow dung, it enhances nutrient quality [31–33]. According to Loh et al. [34], cow dung is a highly favored diet for earthworms across various species. Foods with high microbial composition, good palatability, easy metabolism, and low levels of growth-retarding chemicals favor earthworm growth in waste systems [20]. Therefore, the mixture of brewery sludge with cow dung in this study contributed to positive weight gain and the overall reproductive performance of the species under experimentation. In the present investigation, all experimental earthworm species exhibited continuous biomass increase during the initial weeks of the experiment. However, in the concluding weeks of the study, successive weight loss was observed across all species of earthworms. These progressive weight losses could potentially be associated with a decline in available food, as indicated by Neuhauser et al. [35], and energy expenditure for reproductive activities such as copulation, egg laying, and cocoon formation, as noted by Jesika and Lekeshmanshwaney [36]. Monroy et al. [37] also documented a swift prereproductive phase of growth, succeeded by a stage of gradual biomass and growth reduction upon reaching sexual maturity.

The average time required to attain sexual maturity also varied among the three species. The maturation period for *E. fetida* and *E. andrei*, from the emergence of hatchlings from the cocoon to the appearance of the clitellum, ranged from 21 to 29 days, while for *D. veneta*, it ranged from 42 to 51 days. The maturation time recorded for *E. fetida* and *E. andrei* aligns with the findings of Dominguez [38], who reported an average time to reach sexual maturity in the range of 21–30 days for these species. Additionally, the maturation time obtained for *D. veneta*, 49 to 67 days, align with the standard 65 days for maturation as stated by Dominguez [38]. Shelabi [39] reported a time of 70 days for *E. fetida* and 130 days for *D. veneta* to reach sexual maturity

Table 3: Growth and sexual development of tested earthworm species in brewery sludge mixed with cow dung.

Growth and maturation	Earthworm species		
	E. fetida	*E. andrei*	*D. veneta*
Mean initial weight/worm (g)	0.23 ± 1.65	0.22 ± 1.9	0.31 ± 1.3
Maximum weight attained/worm (g)	0.63 ± 1.58	0.6 ± 1.65	0.94 ± 1.34
Maximum weight achieved on	4th week	4th week	7th week
Average maturation weight	0.592 ± 0.66	0.58 ± 0.76	1.42 ± 0.46
Minimum time to found full clitellum development	4 weeks	4 weeks	7 weeks
Minimum developmental time to start cocoon production	4 weeks	4 weeks	7 weeks

in fecal wastes, which contrasts with the findings of this study. Additionally, Venter and Reineck [28] recorded a maturation period of 60 days for *E. fetida*, which also differs from the present findings.

Food quality, nutrient abundance, and the microbial composition of the feeding substrate are directly associated with the sexual maturity of worms [20, 40]. Therefore, it is plausible to suggest that the relatively short maturation time observed in all experimental worms in this study was linked to the biochemical nature, microbial composition, and nutrient content of the culture material. Similarly, a direct relationship was observed between the weight of the worm and clitellum development in this study. The development of the clitellum in various earthworm species typically initiates at a specific minimum body weight and size [41]. Any factor that impedes growth inevitably results in a delay in reproduction. In the present study, a minimum weight of 0.5 to 0.6 g/worm was recorded for clitellum development in *E. fetida* and *E. andrei*, while in *D. veneta*, a minimum weight between 1 and 1.8 g/worm was recorded. Similar observations have been reported by Dominguez [38].

Additionally, a direct relationship was identified between the body weight and the incubation period of the cocoon in this study. The weight of matured *E. fetida* and *E. andrei* was less than the weight of matured *D. veneta*. Correspondingly, the incubation period of cocoons recorded for *E. fetida* and *E. andrei* was shorter than the incubation period of *D. veneta*. In a similar pattern, Chaudhuri and Bhattacharjee [42] reported a positive association between the body length of various species of earthworms and the incubation period of their cocoons. Consistent with this finding, Podolak et al. [43] reported that the incubation period of *E. fetida* is much faster than that of *D. veneta*. The observed incubation periods of all three earthworm species in this study were also in line with Senapti and Sahu [44] who stated that the incubation period, particularly in temperate worms, ranges from 3 to 30 weeks.

In the current study, all tested species incubated in the experimental substrate exhibited improved hatching success of cocoons, indicating cocoon viability, and a higher number of neonates or hatchlings per cocoon. The number of hatchlings from each viable cocoon of *E. fetida* and *E. andrei* in the experimental substrate aligned with Dominguez [38] findings, who concluded that the number of hatchlings from a viable cocoon of *E. fetida* and *E. andrei* varies from 2.5 to 3.8. However, the hatchling number from each viable cocoon of *D. veneta* in all treatments of this study was lower than the findings of Muyima et al. [45], who recorded a standard hatchling number from each viable cocoon of *D. veneta* at about 1.1.

Since there are limited or no published evidence in the literature concerning the influence of feed quality on cocoon hatchling performance and the number of hatchlings that emerge from a cocoon, it is challenging to establish a direct link between the feeding substrate and these biological activities. However, Suthar [20] suggested that the nitrogen content in the feed substrate might be the main factor influencing cocoon hatching performance. The nitrogen content of the substrate affects the production of cocoons and their incubation by influencing the nutritional requirements of protein for earthworms. Earthworms produce more viable cocoons in nitrogen-rich culture media due to the efficient protein supply in their diets. In contrast to this suggestion, Martin and Lavelle [46] reported that there is no clear relationship between the nitrogen content of the substrate and the reproduction of earthworms.

5. Conclusion and Recommendations

This study assessed the suitability of brewery sludge mixed with cow dung for the survival of three vermicomposting earthworm species *E. fetida*, *E. andrei*, and *D. veneta* in terms of growth and reproductive parameters. Based on the findings, it can be concluded that brewery sludge mixed with cow dung can serve as the growth and reproduction medium for all worm species investigated in this experiment. Therefore, it is feasible to manage brewery waste through vermicomposting by employing suitable worm species. When considering reproductive and growth parameters such as fecundity, incubation period, hatching success, and maturation rate, *E. fetida* and *E. andrei* prove more suitable for vermicomposting brewery sludge mixed with cow dung compared to *D. veneta*.

Data Availability

The data used to support the findings of the study are available upon request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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