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Research Article

Comparative evaluation of growth and reproductive performance of *Eisenia fetida* in various agro-industrial wastes blended with horse manure in Ethiopia

Gezahegn Degefe*
Department of Biology, College of Natural Sciences, Debre Berhan University, Ethiopia
Chrotaw Ayele Gizaw
Department of Biology, College of Natural Sciences, Dilla University, Ethiopia

*Corresponding author. E-mail: gezdeg60@gmail.com

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Abstract
Understanding how different substrates impact the reproductive and growth potential of vermicomposting worms is essential for optimizing their application in waste management across diverse sources and promoting sustainable agriculture. The present study assessed the growth and reproductive performance of *Eisenia fetida*, a key vermicomposting species, across three agro-industrial wastes: brewery malt sludge (W_1), potato chip factory waste (W_2), and avocado oil factory sludge (W_3), mixed with horse manure at a 3:1 ratio. Parameters such as growth, sexual maturation, cocoon production rate, incubation period, and hatching success were evaluated. Results showed cocoon production rates of 0.43 ± 1.22 per worm per day in W1, 0.40 ± 1.44 in W_2, and 0.27 ± 1.62 in W_3. The highest hatchling count per cocoon (3.16 ± 0.54) and maximum weight gain (0.65 ± 0.98 grams) were observed in W_1. Waste from W_1 and W_2 were found to be highly effective substrates for the growth and reproductive performance of *Eisenia fetida*. In contrast, waste from W_3 an avocado oil factory showed comparatively lower performance. No mortality was observed, indicating that these agro-industries could effectively manage their waste through vermicomposting by utilizing appropriate worm species, such as *E. fetida*.

Keywords: Cocoon, *Eisenia fetida,* Epigeic worms, Vermicomposting

INTRODUCTION

Earthworms possess both male and female reproductive organs, making them hermaphroditic, though self-fertilization is rare. Their presence in soil yields positive physical, chemical, and biological impacts, which numerous studies have shown to enhance plant growth and productivity. As the predominant group within soil ecosystems, earthworms are increasingly recognized as ideal candidates for assessing soil fertility and serve as effective bioindicators of soil chemical contamination (Li *et al*., 2018; Parihar *et al*., 2019; Aebeed *et al*., 2022; Ahmed and Al-Mutairi, 2022; Akhia and Entoori, 2022 and Singh and Singh, 2023).
Some species of earthworms are particularly advantageous for the biological conversion of diverse organic wastes into valuable bio-fertilizers through vermicomposting. Species involved in vermicomposting display unique ecological and biological traits. These include a faster consumption of organic matter, efficient digestion and assimilation, and the ability to adapt to various environmental conditions. They also exhibit prolific reproduction, rapid growth, early sexual maturation, and strong resilience to handling and stress. These unique characteristics make them highly suitable for use in vermicomposting activities at different scales (Debnath and Chaudhuri, 2020; Nagannawar *et al*., 2021 and Alipour *et al*., 2022).
Understanding the reproductive and growth efficiency of these worm species in various substrates is essential for optimizing their role in effective waste management systems (Nagannawar *et al*., 2021). Since large volumes of waste can be processed with larger earthworm populations, evaluating the reproductive and growth efficiency of various earthworm species in different wastes can serve as important indicators for as-

sessing their effectiveness in Earthworm-driven biotechnology or vermicomposting (Gezahegn and Girum, 2017). Gaining insights into the life cycle patterns of earthworms is also essential for understanding their population dynamics, productivity, and energy distribution. Comprehending how vermicomposting worms grow and reproduce on various waste materials is essential for effective vermicomposting and vermiculture projects. Various Research works indicate that beyond their intrinsic traits, the development and reproductive success of these earthworms is influenced by the physical and chemical characteristics, taste, and microbial makeup of their food sources (Mahboub et al., 2016; Ali and Kashem, 2018; Sadia et al., 2020).

Ethiopia's agro-based industrial sectors are experiencing significant expansion, which presents substantial environmental challenges due to the waste generated (Alayu & Leta, 2020). Many of these industries discharge large volumes of waste with minimal or no treatment, resulting in considerable environmental harm from the release of inadequately treated or untreated waste into surrounding soil and water bodies (Oljira *et al*., 2018). Given their organic nature, the waste from these industries presents an opportunity to implement environmentally friendly, cost-effective, and innovative biological management techniques, such as vermicomposting, instead of simply disposing of it in landfills.

Although numerous studies have explored the reproductive and growth potential of commonly used earthworm species such as *Eisenia fetda*, *Eisenia andrei*, *Eudrilus eugeniae*, *perionyx excavates*, *Dendrobaena veneta* in vermicomposting across various waste materials (Aynehband *et al*., 2017; Ali and Kassem, 2018; Ananthavalli *et al*., 2019; Prashija and Parthasarathi, 2019; Debnath and Chaudhuri, 2020; Kabi *et al*., 2020; Sadia *et al*., 2020; Shetty and Biradar, 2023; Singh and Singh, 2023 and Gezahegn and Chirotaw, 2024), there is a notable gap in research specifically addressing the life cycle patterns of these worms with regard to agro-industrial wastes typically discarded in Ethiopia. To fill this gap, this study aimed to evaluate how waste materials from a brewery malt factory, a potato chip factory, and an avocado oil factory affect the survival, growth, and reproductive success of *E. fetida*, a key species in vermicomposting.

MATERIALS AND METHODS

This study assessed the reproductive and growth capacity of the vermicomposting earthworm species *E. fetida* by utilizing various agro-industrial wastes. Specifically, sludge from an avocado oil factory, sludge from a brewery malt factory, and solid waste from a potato chips factory were utilized. The experiment was conducted from March 2022 to July 2022 at a vermicomposting farm in Deneba town, Ethiopia. It was carried out in a well-ventilated room utilizing small plastic containers, each with a depth of 12.7 cm and a diameter of 10.16 cm. For effective vermicomposting, the industrial wastes in this study were mixed with horse manure at a ratio of 3:1(Singh and Singh, 2023). A manure fork was used to separate the urine, removing the wetter manure (which had absorbed the urine) from the drier manure (Ferguson & Phillips,2012). Different life cycle parameters of *E. fetida* were studied, such as growth and sexual maturation, cocoon production rate, cocoon incubation period, cocoon hatching success (number of hatchlings).

The experimental wastes were designated as W_1 = brewery malt factory sludge + horse manure, W_2 = solid waste from potato chips factory + horse manure, W_3 = avocado oil factory sludge + horse manure. The experiments were performed at a consistent room temperature of 27°C, with moisture levels regulated between 60% and 70%. Substrate samples were collected every three days to ensure optimal moisture conditions. Each sample was weighed, and then dried at 105^{o}c in an oven for 24 hours. After drying, the samples were cooled in a desiccator for at least an hour before being reweighed. The difference in moisture content was then calculated, and adjustments were made based on the discrepancies between the initial and dried weights (Chauhan *et al*., 2010).

Optimized experimental approach for cocoon production rate (Fecundity) assessment

In this investigation, nine cylindrically shaped plastic containers were used, with three replicates assigned to each type of agro-industrial waste mixed with horse manure. Each container was supplied with sufficient experimental substrate to ensure an uninterrupted food supply during the reproductive phase. Twenty healthy, freshly clitellated *E. fetida* were selected from a vermicompost production center in Deneba, Ethiopia. The worms were first bathed in distilled water to eradicate every adhering substance before being placed into experimental containers containing various agro- industrial wastes. After washing, the worms were fleetingly dried on paper towels and weighed with an electronic balance. Daily assessments were performed for each experimental setup to identify the commencement of cocoon production. Once cocoons were observed, they were manually sorted, briefly cleaned with distilled water, and counted to determine fecundity, represented by the number of cocoons produced per worm per day (Saida *et al*., 2020).

An experimental design for evaluating the incubation period of cocoon, hatching efficiency, and hatchling counts

Twenty newly deposited cocoons were chosen from the fecundity assessment containers to investigate the incubation period (the duration from cocoon deposition to the appearance of the first hatchling), hatching success, and the number of hatchlings per cocoon. The cocoons were transferred into containers containing the same substrate as the parent worms and set up in triplicate. Daily observations were conducted to monitor hatchling emergence and determine the incubation period. At the start of hatching, hatchlings were systematically collected daily with a paintbrush. They were then meticulously sorted and counted to determine the number of neonates emerging from each cocoon per treatment. Concurrently, the number of unhatched cocoons was recorded in all test containers to evaluate hatching performance across the different agro-industrial waste treatments (Ali and Kashem, 2018).

Experimental framework for assessing maturation and biomass dynamics

Biomass change and sexual maturation were investigated using the substrates and containers that were previously utilized for assessing reproductive performance. Each experimental container was supplied with designated test diets to guarantee uniform nutritional conditions. Twenty recently hatched neonates of *E. fetida* were chosen from the containers utilized in the study of cocoon incubation periods. The hatchlings were rinsed with distilled water to eliminate residual substances and gently patted dry with paper towels. They were weighed on an electronic balance before being introduced into the experimental substrates. Over the course of twelve weeks, their growth and biomass changes were monitored weekly through weighing. The study distinguished three specific life stages: the preclitellate stage, identified by the emergence of tubercula pubertatis; the clitellate stage, defined by the presence of a fully formed clitellum; and a subsequent stage in which the clitellum either regresses or disappears. The emergence of successive generations of cocoons was also documented. From the gathered data, several parameters were analyzed, including mean initial weight, maximum weight relative to initial body weight, net weight gained, weight at maturity, time taken to develop a complete clitellum, and the minimum time required to start producing subsequent generation cocoons (Ali and Kashem, 2018).

Statistical analysis

All results are presented as the average of single measurements from each of the three replicates. The data were analyzed using one-way analysis of variance (ANOVA), followed by post-hoc tests to assess significant differences ($p < 0.05$) using the SPSS 29.0 software package. This analysis evaluated variations in growth, cocoon production rates, and the number of hatchlings per cocoon across the three experimental waste treatments.

RESULTS AND DISCUSSION

Numerous investigations have documented how different earthworm species respond to various food sources in both natural habitats and vermicomposting systems (Sonia *et al*., 2016; Ananthavalli *et al*., 2019; Mashur *et al*., 2021; Priya and Sudesh, 2022; Moreno *et al*., 2023 and Maharjan *et al*., 2023). The findings from this study also confirmed that the characteristics of waste materials used in the vermicomposting experiment significantly influence the worms' biomass gain and reproductive success. Notably, no mortality was observed at any stage across all experimental substrates.

Cocoon yield efficiency

The type of feeding substrate significantly influenced the overall rate of cocoon production. When freshly clitellate worms were introduced into the test containers, *E. fetida* in treatments W_1 and W_2 began producing cocoons in the second week, while those in W_3 started production in the third week. Cocoon production persisted through the twelfth week across all experimental substrates. Notably, W_1 (sludge from malt factory + cow dung) showed the highest cocoon production, yielding 742.3 ± 0.82 cocoons at a rate of 0.43 cocoons/worm/day, while W_3 (sludge from avocado oil factory) exhibited the lowest production with 476 ± 0.72 cocoons at a rate of 0.27 ± 1.62 cocoons/worm/day (Table 1). Cocoon production steadily increased across all experimental treatments during the initial eight weeks, followed by a decline in production thereafter. The total number of cocoons generated in W_1 exceeded W_2 by 5.53% and W_3 by 35.84%. Cocoon yields in W_2 surpassed those in W_3 by 32.1%. Significant differences in cocoon production were observed between W_1 and W_3, as well as between W_2 and W_3 ($P < 0.05$).

Various researchers have recorded different numbers of cocoons in their growth and reproductive performance experiments. Chaulagain et al. (2017) found that *E. fetida* grown in a mixture of banana pseudostem and cow dung produced an average of 1.9 cocoons per worm. A combination of leaf litter and cow dung yielded 1.1 cocoons per worm, while a sawdust-cow dung blend resulted in only 0.9 cocoons per worm. Chauhan and Singh (2013) documented 0.052 cocoons per worm per day in rice bran mixed with buffalo dung and 0.097 cocoons per worm per day in a mixture of gram bran and buffalo dung. Podolak *et al*. (2020) documented that *E. fetida* typically produces about 2 cocoons per week, which is a standard measure of its reproductive capacity. In the present experiment, cocoon numbers showed a gradual increase, reaching their peak during the middle phase of the experiment (5th to 8th week). Similarly, in their growth and reproductive experiment, Akhia and Entoori (2022) and Shafique *et al*.

(2023) also observed a gradual rise in cocoon numbers, with the peak occurring during the middle phase of the study.

Incubation duration, hatching rates, and hatchling count per cocoon

The incubation period for *E. fetida* varied based on the substrate. For substrates W_1 and W_2, the period ranged from 3 to 4 weeks, whereas for W_3, it extended from 4 to 5 weeks. Substrate W_1 exhibited the highest hatchling count, averaging 63 ± 0.81 hatchlings, or 3.16 hatchlings per cocoon. In contrast, substrate W_3 had the lowest hatchling yield, with an average of 43.8 ± 0.37, corresponding to 2.15 ± 0.86 hatchlings per cocoon (Table 2). A significant difference was observed between W_1 and W_3 ($p < 0.05$) as well as between W_2 and W_3 ($p < 0.05$); however, no significant difference was found between W_1 and W_2 ($p > 0.05$). Upon emergence, the hatchlings were white and unpigmented, gaining pigmentation within a few days. Overall, cocoon hatching performance was highest in W_1 and W_2, while W_3 proved to be the least effective.

In the present study, *E. fetida* showed enhanced cocoon hatching success across all tested substrates, demonstrating improved viability of cocoons and a larger number of hatchling or neonates per cocoon. While there is limited literature on the direct impact of the nature of substrate on cocoon hatching performance, Kabi *et al*. (2020) mentioned that nitrogen richness in the substrate may play a crucial role. The nitrogen content influences the rate of cocoon production and subsequent growth by meeting the protein requirements of worms. It could be proposed that earthworms yield more viable cocoons in nitrogen-abundant environments, because of the superior protein availability in their diets (Biradar and Amoji, 2023).

Growth, sexual maturation, and cocoon initiation

Following the introduction of newly hatched hatchlings into the experimental substrate, those in W_1 and W_2 exhibited a consistent increase in biomass. This trend continued until the fifth week, after which their growth stabilized until the ninth week. In contrast, hatchlings in W_3 demonstrated continuous growth until the sixth week, after which their growth stabilized until the ninth week. In W_1, where maximum growth occurred, *E. fetida* reached its peak weight of 0.65 ± 0.98g per worm by the 4th week (Table 3). The maximum weight recorded across all substrates showed a significant deviation from the initial weight at the start of the experiment ($P < 0.05$). After the ninth week, the biomass of worms in each experimental container gradually decreased.

The sexual maturity of the worms was assessed by the presence of a prominent and engorged clitellum. After introducing hatchlings into the test substrate, the first preclitellate worms, marked by the appearance of tubercula pubertatis, emerged in W_1 and W_2 by the third week and W_3 by the fourth week. The first sexually mature worms, characterized by fully developed clitella, were detected in W_1 and W_2 by the fourth week and W_3 by the fifth week. *E. fetida* began to release cocoons in W_1 and W_2 during the fifth week, while in W_3, cocoon production started in the sixth week. The shortest time required for maturation, from hatchling emergence to full clitellum development, was four weeks in W_1 and W_2 and five weeks in W_3. Moreover, the average time required to complete the life cycle from the deposition of a new cocoon, through incubation and clitellate worm maturation, to the emergence of the next generation of cocoons varied between seven and eight weeks in W_1 and W_2 and extended to nine weeks in W_3.

The findings from this study also underscore that the characteristics of waste materials utilized in vermicomposting and vermiculture can greatly affect the biomass gain and reproductive success of vermicomposting worms. In general no mortality was observed at any stage across all experimental substrates. In this study the most significant gain in the biomass of the worm occurred in W_1 and W_2, while W_3 showed the smallest growth. This variation is probably caused by the physi-

Table 1. Cocoon production of *Eisenia fetida*

Treatment	W_1	W_2	W_3
Cocoon production started at	2nd week	2nd week	3rd week
Average cocoon yield	742.3 ± 0.82	701± 0.62	476± 0.72
Cocoon output per worm	37.1± 1.42	35± 0.92	23.8± 1.32
Average weekly cocoon yield per batch	61.8± 1.42	58.5 ± 1.63	39.6 ± 0.82
Average weekly cocoon production per worm	3.1± 1.33	2.9 ± 1.37	1.86± 1.45
Average daily cocoon yield per worm	0.43± 1.22	0.42± 1.44	0.27 ± 1.62
Time span until cessation of cocoon production	12th	12th	12th

W_1 = brewery malt factory sludge + horse manure, W_2 = solid waste from potato chips factory + horse manure, W_3 = avocado oil factory sludge + horse manure

Table 2. Incubation periods and hatching success rates of *Eisenia fetida*

Experimental treatment	W_1	W_2	W_3
Duration of incubation in days	21-27	21-27	27-33
Total count of hatched cocoons	19.5 ± 0.68	19± 0.88	15± 0.88
Success rate of hatching (%)	97.5	95	75
Total number of hatchlings that emerged	63± 0.81	57.7± 0.53	43.8± 0.37
Ratio of hatchlings per cocoon	3.16± 0.54	2.87± 0.88	2.15 ± 0.86

W_1 = brewery malt factory sludge + horse manure, W_2 = solid waste from potato chips factory + horse manure, W_3 = avocado oil factory sludge + horse manure

Table 3. Growth and sexual development of *Eisenia fetida*

Growth parameters	W_1	W_2	W_3
Average initial weight of each worm (g)	0.23 ± 1.35	0.23 ± 1.43	0.23 ± 0.85
Maximum weight attained by each worm (g)	0.65 ± 0.98	0.64 ± 1.04	0.62 ± 1.03
Week on which peak weight was achieved	4^{th} week	4^{th} week	5^{th} week
Average weight at maturity	0.59 ± 0.66	0.61 ± 0.76	0.58 ± 0.66
Shortest time needed for complete clitellum development	3 weeks	3 weeks	4 weeks
Minimum time required for the onset of cocoon production	5 weeks	5 weeks	6 weeks

W_1 = brewery malt factory sludge + horse manure, W_2 = solid waste from potato chips factory + horse manure, W_3 = avocado oil factory sludge + horse manure

cal as well as biochemical attributes of the feeding substrate, such as nitrogen content, microbial composition, palatability, substrate metabolizability, and low levels of growth-inhibiting substances, which play a crucial role in worm growth as well as the time required to achieve sexual maturity (Ali and Kassem, 2018; Jayakumar *et al*., 2018; Sadia *et al*., 2020).

Throughout the experiment, *E. fetida* in all experimental containers initially showed consistent weight gain, followed by a progressive weight decline as the study concluded. This trend mirrors findings from (Mahboub khomami *et al*., 2016; Ali and Kashem, 2018 Debnath and Chaudhuri, 2020; Shafique *et al*., 2023), who observed a rapid growth phase followed by a gradual decrease in biomass in several vermicomposting species in various kinds of wastes. The decrease in worm biomass observed could be due to food resource depletion. Vermicomposting worms tend to lose weight when food is insufficient for maintenance (Bhat *et al*., 2016 and; Sonia *et al*., 2016). Moreover, weight loss may also be associated with worms reaching maturity, during which energy is redirected towards reproductive processes like copulation, egg- laying and cocoon production (Sadia *et al*., 2020).

The maturation period(full clitellum growth) observed in the present study aligns with the findings of Ali and Kashem (2018), who reported that *E. fetida* requires about three to four weeks to develop a fully formed clitellum. Several studies have recorded different maturation times for *E. fetida* in diverse substrates. Gezahegn and Girum (2017), Podolak *et al*. 2020, and Gezahegn and Chirotaw (2024) found that *E. fetida* developed clitella within 15, 30, and 28 days in various organic wastes, respectively. The time required for sexual maturity is closely associated with the nutrient richness, diet quality, and the microbial composition of the substrate (Jayakumar *et al*., 2018). Therefore, it can be hypothesized that the comparatively longer maturation period observed in W_3 in this study might be attributed to the biochemical properties of the substrate, though this hypothesis requires additional experimental validation.

It is well-established that growth is crucial for organisms to reach maturity and begin reproduction; any interruptions in growth can affect an individual's entire lifecycle. This study observed a positive correlation between the weight of *E. fetida* and the development of its clitellum. In W_1, W_2, and W_3, *E. fetida* required a minimum weight of 0.5 to 0.6 g/worm for clitellum formation. This observation is consistent with the findings of Podolak et al. (2020) and Gezahegn and Chirotaw (2024), who established a direct link between the weight of vermicomposting worms (*E. fetida*, *E. andrei*, and *D. veneta*) and the appearance of the clitellum, indicating sexual maturity.

Conclusion

This study investigated the effectiveness of three types of agro-industrial waste namely, sludge from a brewery malt factory, waste from a potato chips factory, and sludge from an avocado oil factory, each mixed with horse manure to enhance the reproductive and growth capabilities of the vermicomposting species *E. fetida*. The findings demonstrated that all three agro-industrial wastes can serve effectively as substrates to stimulate growth and reproduction in these earthworms. Specifically, in terms of reproductive parameters such as cocoon production rate, incubation period, and hatching performance, *E. fetida* exhibited superior performance when exposed to sludge from the brewery malt factory and waste from the potato chips factory, as compared to sludge from the avocado oil factory. This variation is probably caused by the physical as well as biochemical attributes of the feeding substrate, such as nitrogen content, microbial composition, palatability, substrate metabolizability, and low levels of growth-inhibiting substances, which play a crucial role in worm growth as well as the time required to achieve sexual maturity. Therefore, utilizing *E. fetida* in vermicomposting represents a feasible strategy for managing these agro-industrial wastes.

Conflict of interest

The authors declare that they have no conflict of interest.

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