RESEARCH ARTICLE

Optimizing vermicomposting: *In situ* monitoring of *Eisenia fetida* (Savigny, 1826) reproduction dynamics at SAIN farm, Southern Benin

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Vermiculture, an agroecological technique, provides a sustainable alternative to chemical inputs for soil fertilization. While the reproduction dynamics of *Eisenia fetida*, a key species for vermiculture, have been extensively studied in laboratory settings, gaps remain in our understanding of their behavior under real-world conditions. This study aimed to assess the reproductive performance of *E. fetida* in actual vermicomposting units at Sustainable Agriculture and Innovation Network (SAIN) farm, Southern Benin. Fifteen vermicomposting units, each containing 5 kg of fresh cattle manure and 30 g of *E. fetida*, were established with three substrate supplementation frequencies: monthly, bimonthly and quarterly. Physicochemical parameters of the substrate and biological parameters of the worms were monitored over a three-month period. Results indicate that the population and biomass of *E. fetida* doubled within a month, with a peak in reproduction observed after two months. However, significant substrate acidification was noted, potentially impacting substrate quality and worm growth. These findings suggest that initial density and substrate quality are critical factors for optimizing *E. fetida* reproduction. Future research should explore the effects of different initial densities and evaluate various organic materials to improve substrate quality. Such studies could contribute to the adoption of more sustainable agricultural practices, particularly in regions transitioning away from chemical inputs.

Key Words: *Vermicompost; Sustainable agriculture; Substrate quality; Benin*

INTRODUCTION

Vermicomposting is an agroecology technique with the potential to partially replace chemical fertilizers for soil fertilization [1-4]. It offers several advantages over traditional composting, notably because it contains more nutrients, microorganisms and growth regulators for plants, such as auxins and cytokinins [1,5]. Research has shown that the use of vermicompost as a base fertilizer accelerates plant maturation by 1 to 2 weeks and enhances soil enzymatic activity [5]. For its production, cattle manure, such as that from *Bos taurus indicus*, is commonly used [5-7]. The most frequently used earthworms are *Eisenia fetida* (manure worm), *Eisenia andrei* (red wiggler) and *Dendrobaena venata* (European nightcrawler) [1,5,6,8]. The castings of *Eisenia fetida* are rich in nitrates, providing bioavailable forms of potassium, calcium, magnesium and phosphorus. This agroecological technique thus promotes a sustainable approach to agriculture by preserving biodiversity and enhancing soil functionality [7,8].

Literature indicates that the biomass and population of *Eisenia fetida* can double within a month under controlled laboratory conditions [1,7,8]. However, *in situ* conditions introduce predators like *Hemidactylus frenatus* (house gecko) and *Oecophylla longinoda* (weaver ant), particularly in West Africa. These ants also consume the food intended for the worms. To prevent their presence, it is recommended to maintain a neutral or slightly basic pH [2].

To better understand the biological cycle of *Eisenia fetida* in *in situ* vermicomposting, this study aims to investigate: (i) population growth; (ii) biomass increase; (iii) the impact of monthly observations on reproductive cycles; (iv) the influence of substrate supplementation on *Eisenia fetida* growth.

MATERIALS AND METHODS

Study area

The study was conducted at the SAIN farm in Kakanitchoé, located in the commune of Adjohoun, Ouémé department, Benin (Figure 1). Adjohoun, situated 32 km north of Porto-Novo, has a population of approximately 75,323 inhabitants and spans an area of 308 km^2. The region's subtropical climate features two rainy seasons and two dry seasons, with an annual rainfall averaging 1,112.19 mm over 50 days, posing challenges for rainfed agriculture (Ministry of Agriculture, Livestock and Fisheries of Benin, 2014). The topography includes a low-altitude plateau and a floodplain along the Ouémé River, resulting in two primary soil types: Fertile lowland soils ideal for rice and vegetable farming, and poor, leached ferralitic soils with low productivity. These soils are further degraded by intensive agriculture and bushfires (Ministry of the Environment and Sustainable Development, 2015). The experiment was conducted during the main growing season from April to September, which is the intensive farming period for cucumber in this region.

Species description: *Eisenia fetida*

Eisenia fetida, commonly known as the "manure worm" or "red wiggler", is an annelid species belonging to the family Lumbricidae. This species is highly valued in vermiculture due to its exceptional ability to break down organic matter and produce nutrient-rich vermicompost. *Eisenia fetida* is characterized by its remarkable resilience and its ability to thrive in various environments rich in decomposing organic matter, such as composts and manures [8]. Adult worms typically range from 5 to 12 cm in length and can live for several years under optimal conditions [9]. Their rapid reproduction rate, with populations capable of doubling in just 30 to 45 days under favorable conditions, makes them ideal for large-scale vermicomposting [1]. *Eisenia fetida* is also known for its tolerance to a wide range of temperatures and humidity levels, although it prefers moderate conditions with a relative humidity of 70%-80% and temperatures around 20°C-25°C [10]. Additionally, this worm species can bioaccumulate certain heavy metals, making it a potential bioindicator for contaminated soils (Figure 2) [11].

Experimental design

The experimental setup was based on the vermicomposting conditions at the SAIN farm in Kakanitchoé [1-3]. Fifteen identical plastic boxes (40 × 20 × 20 cm) with drainage holes were used as vermicomposting units. Each box was filled with 5 kg of fresh cattle manure (*Bos taurus indicus*). After a 4-day pre-composting period to reduce ammonia levels, 30 grams of *Eisenia fetida* were introduced into each box on the fifth day. To maintain humidity between 70% and 80%, which is most favorable for the reproduction of this species, 3 liters of water were added twice a week (on Mondays and Thursdays). The experiment was conducted using a completely randomized design, with three

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treatment groups, each consisting of five replications. Sampling was carried out at three time points: T1 (1 month), T2 (2 months) and T3 (3 months). At each sampling point, a composite sample of 75 ml was taken from each box by collecting sub-samples from the upper, middle and lower layers.

Monthly supplementation (1 M): The substrate was turned and supplemented with 20% fresh manure every month.

Bimonthly supplementation (2 M): The substrate was turned and supplemented with 20% fresh manure every two months.

Quarterly supplementation (3 M): The substrate was turned and supplemented with 20% fresh manure every three months.

Laboratory analyses

The physicochemical parameters analyzed included moisture content, pH (H_2O) and the concentrations of nitrogen, phosphorus and potassium. To obtain these measurements, a composite sample of 75 mL was taken from each box using a cross-sectional sampling method across the entire substrate depth. Field probes with a 2% margin of error were used to determine the concentrations of these elements. The pH (H_2O) was measured according to the NF ISO 10390 standard using a pH meter. Moisture content was measured according to the NF ISO 11465 standard. For each sample, 15 mL was dried at room temperature in the shade for 10 days, placed in a beaker and 25 mL of distilled water was added. The preparations were then agitated for 30 min. Afterward, 10 mL of the composite was weighed, dried and weighed again. This allowed for the determination of the moisture content (H) using the following formula:

$$H = \frac{(mf - ms)}{mf} \times 100$$

Where, H is the moisture content (%), mf is the fresh mass of the sample (g), ms is the dry mass of the sample (g).

The biological parameters monitored included the mass of *Eisenia fetida* individuals, their number and the number of newborns. For this, during each sampling, the worms were manually counted, placed in a beaker and weighed.

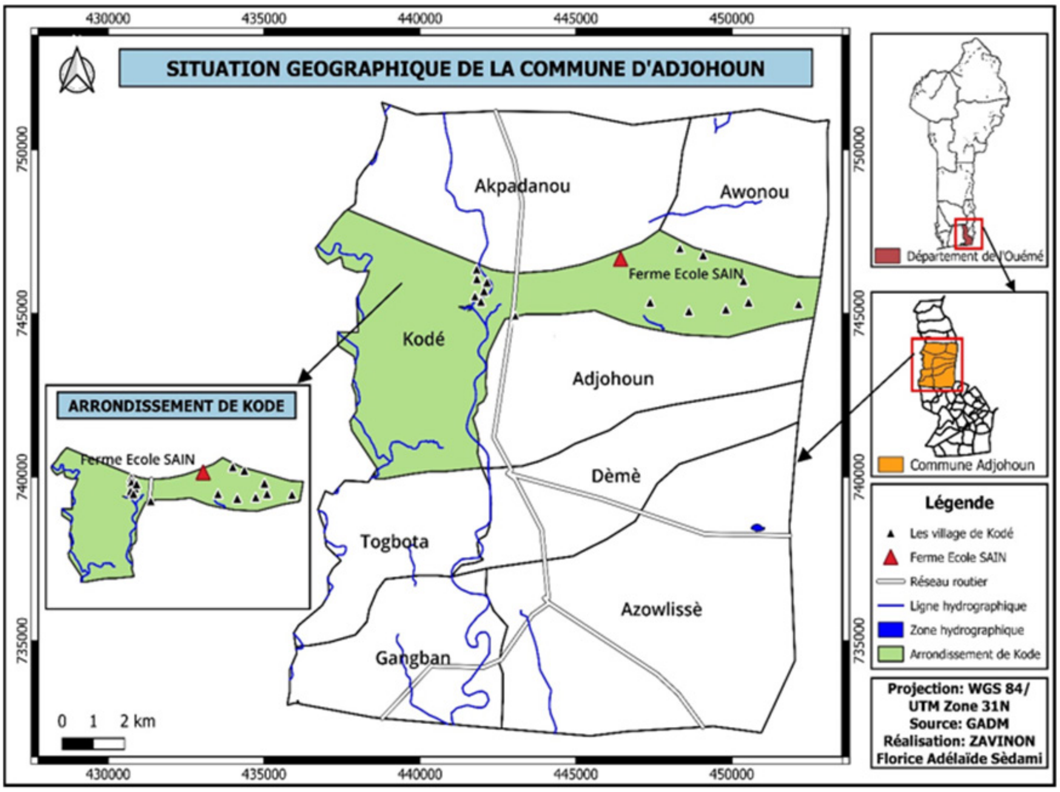

Figure 1) *Study area showing the geographical location of commune of Adjohoun in district of Kode, Benin*

Figure 1) *Images of Eisenia fetida (worms) in vermi composting process*

Statistical analysis

All statistical analyses were performed using the R software (version 4.2.1). For the 1 M treatment, when the normality of the residuals and homoscedasticity were validated, differences in each parameter were tested using Analysis of Variance (ANOVA). If these conditions were not met, a Kruskal-Wallis test was conducted. Post hoc Honestly Significant Difference (Post hoc HSD) Tukey tests were then applied for multiple comparisons. For the 2 M and 3 M treatments and the comparisons between 1 M/2 M and 1 M/3 M, a normality test of the residuals was performed for each variable. If normality was respected, a Welch test was conducted. If not, a Mann-Whitney test was performed.

RESULTS AND DISCUSSION

Evolution of biological parameters of *Eisenia fetida* over 3 months for the 1 M modality

The species *Eisenia fetida* exhibited significant population growth (number of individuals) under the 1 M modality over the 3-month experiment (Table 1). The individual mass also significantly increased between T0 and T3 (Tukey test, $p<0.05$). Newborns were more numerous at T2 and T3 compared to T0 (Tukey test, $p<0.05$). In the first month, the mass increased by 134 g, in the second month by 82 g, and in the third month by 238 g. The number of individuals increased by 51% in the first month, though this change was not statistically significant. However, in the second month, the number increased significantly by 933% compared to the first month. In the third month, no significant difference in the number of individuals was observed compared to the second month. Finally, for the number of newborns, a significant increase was observed in the second month, with 9282 additional individuals compared to the previous month. However, in the third month, the number of newborns decreased to 22, with no significant difference compared to T0 and T1.

Comparison of analyzed parameters between modalities

For the three sampling periods (T1, T2 and T3) and across all three treatment modalities, the concentrations of nitrogen, phosphorus and potassium were significantly lower compared to the end of the pre-composting phase (Table 2). During these same periods, no significant differences in these concentrations were observed for the 1 M modality. Regarding moisture content, no significant differences were noted and it remained between 70% and 80%. As for pH, significant acidification was observed after two months for the 1 M modality, with similar results for the 2 M and 3 M modalities.

When comparing the 1 M and 2 M modalities after two months and the 1 M and 3 M modalities after three months, no significant differences were observed in nitrogen, phosphorus, potassium concentrations, moisture, the number of individuals and the number of newborns (Tables 2 and 3). However, the pH was significantly more acidic for the 2 M and 3 M modalities compared to the 1 M modality. The worm mass was significantly higher for the 2 M modality compared to the 1 M modality (836 g) (Table 2). Conversely, it was significantly lower for the 3 M modality compared to the 1 M modality (65 g) (Table 3).

The results presented in this study provide a detailed overview of the evolution of the physicochemical parameters of the substrate and the population dynamics of *Eisenia fetida* during the vermicomposting process. By comparing these results with current knowledge on the subject, we aim to understand the underlying mechanisms of these changes and identify factors that influence the performance of the vermicomposting system. Although this study offers interesting insights, it is important to highlight certain limitations related to the experimental protocol that could affect the interpretation of the data.

Regarding the physicochemical parameters, the moisture content was successfully maintained at a level favorable to the reproduction of *Eisenia fetida* individuals [1]. The observed acidification of the pH can be explained by the mineralization of nitrogen and phosphorus compounds, along with the production of organic acids such as humic and fulvic acids during the vermicomposting process [12]. With the substrate used (*Bos taurus indicus* manure), a significant increase in nitrogen, phosphorus and potassium concentrations is expected after 45 days of vermicomposting [13-15]. However, in this experiment, no measurements were taken at this time point as it was not the objective. It is therefore assumed that these concentrations significantly increased for the treatments after 45 days. This assumption implies that they subsequently decreased. However, no study supports this hypothesis. It is also possible that these results are due to the lack of precision of the N, P and K probes used.

TABLE 1

Evolution of biological parameters (mass, number of individuals, newborns) of *Eisenia fetida* over 3 months for the 1 M modality

Time (month)	Number of worms	Number of newborns	Mass of worms (g)
T0	65,6[c]	0[c]	30[c]
T1	99,4[b]	33,8[b]	164[b]
T2	1027,6[a]	928,2[a]	246[a]
T3	1029,8[a]	2,2[c]	269,8[a]

Note: Means followed by same letter are not significantly different according to the honestly significant difference Tukey tests; T0: 04/03/2024 (Initial introduction of 30 g of worms); T1: 02/04/2024 (1 month); T2: 30/04/2024 (2 months); T3: 28/05/2024 (3 months).

TABLE 2

Comparison of analysed parameters between 1 M and 2 M modalities

Sample	Mass (g)	Number of worms	Newborns	Nitrogen (mg/L)	Phosphorus (mg/L)	Potassium (mg/L)	Moisture (%)	pH (H_2O)
1 M	246 ± 11.66	1027.6 ± 114.8	1027.6 ± 114.80	16.4 ± 4.51	15.6 ± 6.69	37.2 ± 6.57	78.48 ± 2.9	7.06 ± 0.3
2 M	329.6 ± 57.2*	1195.4 ± 244.4	1123.2 ± 246.4	19.4 ± 4.51	21.4 ± 2.97	50.8 ± 16.18	77.9 ± 5.2	6.72 ± 0.04*

Note: (*): Indicate significant differences between the two modalities (p-value<0.05).

TABLE 3

Comparison of analysed parameters between 1 M and 3 M modalities after 3 months

Sample	Mass (g)	Number of worms	Newborns	Nitrogen (mg/L)	Phosphorus (mg/L)	Potassium (mg/L)	Moisture (%)	pH (H_2O)
1 M	269.8 ± 24.16	1029.8 ± 179.60	1029.8 ± 179.60	26.2 ± 5.54	23.8 ± 6.14	65.2 ± 13.22	82.72 ± 6.28	6.92 ± 0.3
3 M	204.8 ± 15.85*	836.2 ± 172.67	770.6 ± 182.63	38.6 ± 10.01	38.2 ± 12.52	93.6 ± 27.68	86.6 ± 2.30	6.58 ± 0.04*

Note: (*): Indicate significant differences between the two modalities (p-value<0.05).

Regarding the number of individuals, the population doubled within one month, as indicated in the literature [1,7,8]. However, the significant increase of 933% occurred in the third month. In tropical environments, reproduction can be very high and rapid [16]. Based on the number of individuals at T2 and T3 and the significant decrease in the number of newborns at T3, the worm population appears to reach a plateau after two months. However, for this species and substrate, the most favorable density is 10 worms per 52 cm^3 [17]. Therefore, the number of individuals should be able to increase further to reach 53,790. Previous research compared a density of 10 (10 per 52 cm^3) with a density of 30 (30 per 52 cm^3), highlighting that space and food availability limited intraspecific competition, leading to a higher reproduction rate [17,18]. The difference with our experiment may be due to the experimental conditions. In the cited studies, vermiculture beds were used.

According to this experiment, the reproductive cycles were not disrupted by the measurements, as the number of newborns and individuals did not significantly differ between the treatments.

The evolution of the worms' mass is consistent with the literature. First, for the 1 M treatment, the mass doubled every month, confirming our hypothesis [1]. Additionally, Djossa and Houndonougbo found an average mass of 15 g per individual after 8 weeks. After 2 months, we found an average mass of 166 g for the 1 M treatment. However, for the 2 M treatment, the average mass of an individual was 0.28 g. This may be explained by the 20% substrate supplementation in the first month for 1 M but not for 2 M. Finally, the significant difference in mass between the 1 M/2 M and 1 M/3 M treatments can be attributed to the "quality" of the substrate. It has been shown that the nutritional value of the manure used as a substrate is a major factor in biomass evolution [17,18]. The hypothesis is that the nutrient concentration in the substrate was higher in the 2 M treatment compared to the 1 M treatment and lower in the 3 M treatment compared to the 1 M treatment.

CONCLUSION

This study has confirmed that *in situ*, the population and biomass of *Eisenia fetida* double within one month. The results also indicate that the highest reproduction rate occurs at the two-month mark, reaching a plateau thereafter. However, further research is necessary to identify the optimal initial density that maximizes reproduction rates.

To enhance the reproduction rate, additional factors must be considered, particularly the quality of the substrate, as determined by its nutrient concentration. Moreover, to mitigate acidification of the environment and address the challenge posed by *Oecophylla longinoda* (weaver ants), an experiment incorporating eggshell supplementation could be conducted to maintain a neutral pH level.

Future studies should also explore the long-term effects of substrate supplementation on worm population dynamics and evaluate different organic waste materials as substrates to optimize vermicomposting processes. These insights could significantly contribute to the development of more sustainable and efficient agricultural practices, particularly in regions where chemical inputs are being reduced.

AUTHOR CONTRIBUTIONS

Pascal Gbenou, Charlotte Zoundji and Lucile Ghis: Conceptualization, data collection, data analysis, article writing and its correction.

Ibouraïman Balogoun and Charlotte Zoundji: Supervision, editing and validation before submission.

Pascal Gbenou: Supervision and validation. All authors reviewed the results and approved the final version of the manuscript.

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