



Morphological Characterization of Two Categories of Earthworms under the Agroecological Conditions of Mali

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Abstract

This study, conducted in Badougou Djoliba, aimed to investigate the optimal conditions for vermicomposting and to characterize the morphological traits of two earthworm species: the exotic or manure worm, *Eisenia foetida*, and the local strain, *Eisenia eugeniae*, within the agropedological context of Mali. Manure was collected from domestic livestock on peri-urban farms in Bamako. Two experimental setups were implemented: one in a natural environment and the other under controlled conditions. During the vermicomposting process, three individuals of each species, *E. foetida* and *E. eugeniae*, were introduced into the pre-compost bins for 40 days. Five substrates were tested for vermiculture: 100% urban waste, a mixture of 5% poultry manure and 95% urban waste, a combination of 2/3 plant waste and 1/3 urban waste, a mixture of 1/3 plant waste and 2/3 urban waste, and bare soil. Additionally, a control group without earthworms was included. The results indicated that the viability of the worms in the small ruminant manure mixed with soil was 100% for the proportions FPR-1/1, FPR-1/3, and FPR-1/4. In contrast, a viability rate of 67% was observed in the FPR-1/0 proportion. In the poultry manure mixed with soil, a viability rate of 0% was recorded for the FPR-1/1 proportion, resulting in three deaths. However, a viability rate of 67% was noted for the FV-1/3 proportion, with only one mortality, while the FV-1/4 proportion showed a 100% viability rate, with no deaths reported. In terms of vermiculture, a 100% viability rate was observed for both *E. eugeniae* and *E. foetida* in the 100% urban waste treatment. Similarly, a viability rate of 100% was achieved for both earthworm species in the 2/3 plant waste and 1/3 urban waste mixture. *E. eugeniae* also exhibited a 100% viability rate in the 1/3 plant waste and 2/3 urban waste mixture. This study successfully characterized the observable, structural, and measura-

ble morphological traits of the earthworm species by analyzing their shape, structure, and physical variability in an agro-pedo-ecological environment.

Subject Areas

Agricultural Sciences

Keywords

Morphological Characterization, Earthworms, Agro-Pedo-Ecology, Mali

1. Introduction

In the Sudano-Sahelian region, earthworms, which reside permanently in the mineral layers of the soil, are one of the most significant sources of biomass and play a crucial role in maintaining soil health. These organisms serve as a highly nutritious ingredient in poultry and livestock feed, effectively enhancing soil aeration, structure, stability, and nutrient availability across various soil layers. Earthworms have increasingly gained importance due to their multifaceted roles in the economy, environment, agronomy, and animal husbandry, making them a valuable asset in organic waste management, agricultural land fertilization, and animal feed production [1] [2].

As a natural source of animal protein, earthworms contain approximately 63% protein, which is vital for the growth, health, and egg production of poultry. By residing in the soil, they stimulate foraging activity, thereby enhancing the well-being of birds while supplying essential amino acids, lipids, and minerals. Furthermore, endogeic earthworms contribute significantly to their environment through various physicochemical and biological mechanisms, which improve soil fertility and preserve soil structure. They represent a substantial portion of soil animal biomass, playing a central role in maintaining soil functioning [3].

Research indicates that around 5000 species of earthworms have been described, making them a major component of terrestrial animal biomass worldwide; however, many species, particularly in tropical regions, remain undiscovered [4]. Among these, the species *E. foetida* is frequently recognized in studies as the most effective in the waste bioconversion process [5]. Distinct characteristics define each species; for instance, *E. foetida* is the smallest, measuring 90 mm in length and exhibiting colors that range from red to green to brown [6]. In contrast, *E. eugeniae* measures between 130 and 150 mm in length, has a dark red body, and contains 145 to 207 body segments [7].

Earthworms also fulfill critical roles within agroecosystems by influencing the physical, chemical, and biological dynamics of soil through three primary functions: the creation of burrows and the formation of casts, which are characteristic features of endogeic and anecic earthworms [8]. Endogeic earthworms inhabit most terrestrial ecosystems, especially in temperate and tropical regions, with *E.*

eugeniae holding a notable economic significance in poultry farming [4].

Soil is home to one of the most complex assemblages of living organisms, which engage in interactions with both the organic and inorganic components of the soil. Among the biotic components, terrestrial invertebrates are essential players in these interactions, significantly impacting the physical, chemical, and biological characteristics of soil and ensuring its capacity to provide vital ecosystem services [9].

This study aims to undertake a morphological characterization of two categories of earthworms under the agroecological conditions of Mali.

2. Materials and Methods

The animal research ethics committees of the École Doctorale des Sciences et Technologies du Mali and the Laboratoire d'Entomologie et Parasitologie, USTTB-FST have approved this research protocol. This approval is in line with the guidelines established by both academic and research institutions.

2.1. Experimental Site

The study was carried out in Badougou Djoliba (Figure 1) in collaboration with the Institut Polytechnique Rurale de Formation et de Recherche Appliquée de Katibougou, the Laboratoire d'Entomologie et Parasitologie at USTTB-FST, and the École Doctorale des Sciences et Technologies du Mali.

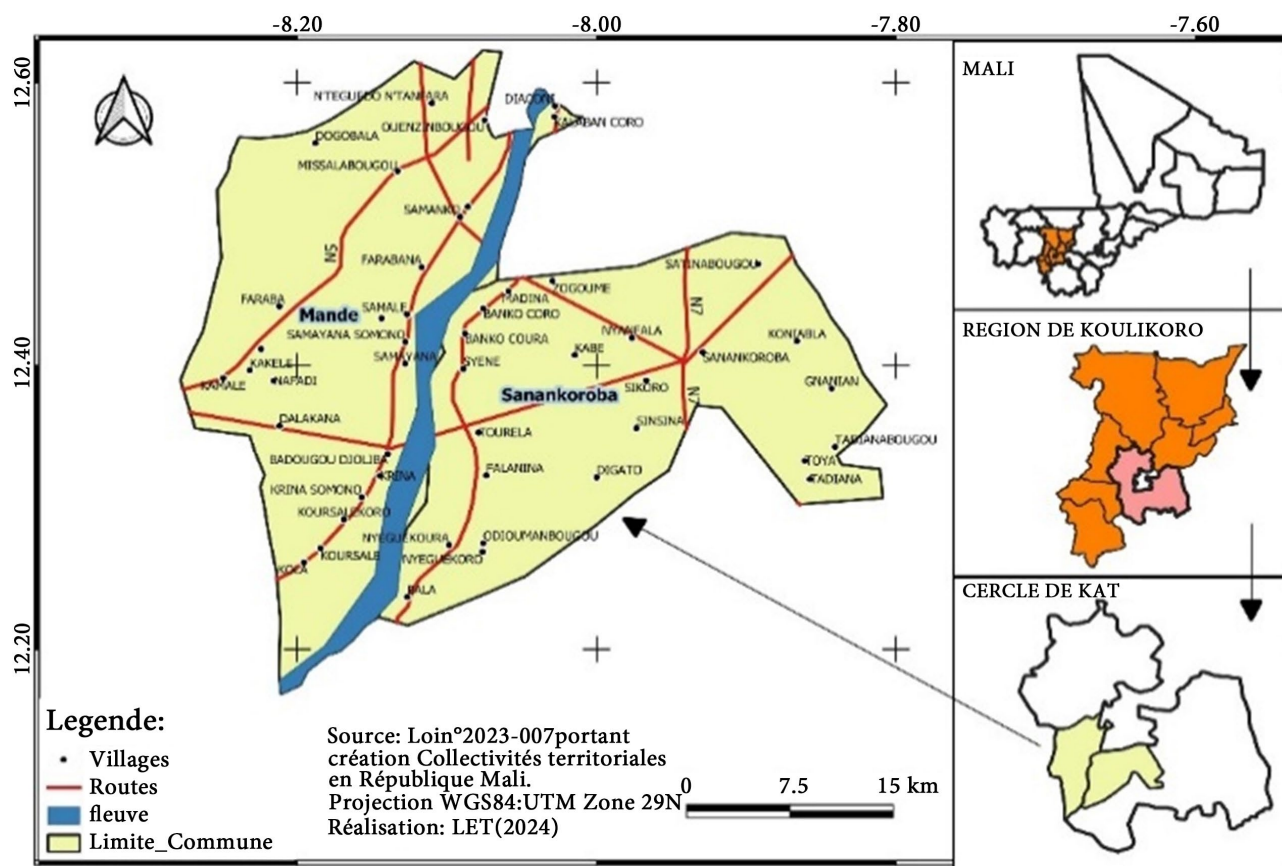


Figure 1. Map of the study area.

Biological and technical materials were used to conduct this research. The experimental site was surveyed to identify areas for earthworm collection, which was facilitated by the presence of casts on the soil surface.

2.2. Biological Materials

The biological materials included earthworms, indigenous epigeic worms, and manure worms.

2.3. Technical Equipment

Earthworms were identified and characterized through the morphological observation of their external features. For this, we used binocular magnifying glasses, tweezers, a precision electronic scale, a ruler, alcohol, a camera, Petri dishes, data sheets, and pens.

3. Methods

3.1. Sampling and Collection of Earthworms

Earthworm sampling primarily uses chemical methods for anecic species and physical methods for endogeic and epigeic species.

The sampling took place at the experimental site during the rainy season, from June to July 2024. Earthworms were collected following the methods outlined by [10], which involved digging at the sampling point, manually sorting through manure piles, or examining the surface layers of the soil (up to 15 cm deep) in areas known to harbor earthworms. This was done to minimize biotype variation within the same plot of land.

The earthworms were collected three weeks before the experiment began (Figure 2). After collection, the samples were stored in a cool place in containers filled with moist soil from the sampling site, taking into account the soil's physicochemical properties.



Figure 2. Sampling and collection of earthworms on site.

3.2. Pre-Composting

Pre-composting is an initial stage in the accelerated decomposition of organic matter that occurs before final composting or vermicomposting. This process

can reduce waste volume by nearly 60%, minimize odors, and sanitize the material.

In this study, traditional pre-composting was essential before introducing earthworms (**Figure 3**). This phase, characterized by high heat generation, achieved sufficient temperatures to eliminate most pathogens, prevent harmful fermentation that could impede worm development [11]. And sanitize the material.



Figure 3. Different types of pre-compost.

3.3. Assessment of Earthworm Viability in Different Substrates

A test was conducted to evaluate the viability of earthworms based on the ratio of pre-compost to soil. This test utilized the optimal compost-to-soil ratio for promoting earthworm development, focusing on their survival, growth, and reproduction across various substrates, including soil, compost, and manure.

The viability of the earthworms in these different substrates was assessed using a specific formula.

$$\text{Viability observed} = \frac{\text{Number at the end}}{\text{Initial number}} \times 100$$

Three types of pre-compost manure were utilized. For each type of manure, we prepared four different percentages, varying from raw manure to a mixture of one part pre-compost and four parts soil (**Table 1**).

Table 1. Substrate report for the viability test.

Manure/soil pre-composts	Report
Pre-compost/soil T0	1/0
Pre-compost/soil T1	1/1
Pre-compost/soil T2	1/3
Pre-compost/soil T3	1/4

Each container was filled with substrate (pre-compost/soil from the experimental site) and three earthworms.

3.4. Setting Up of Experimental Apparatus

Two types of studies were conducted: one in a natural environment and the other under controlled conditions.

3.4.1. Field Tests of Vermicomposting

Vermicomposting is an eco-friendly method that breaks down organic waste into compost using macro- and microorganisms, such as bacteria and worms. The experiments were conducted in rural areas with animal waste to determine the environmental conditions conducive to vermicomposting.

Experimental Setup:

The experimental setup consisted of specific materials, experimental units, and organizational arrangements. Trenches measuring 40 cm wide, 50 cm long, and 10 cm deep were dug. The spacing between the trenches was 60 cm in width and 1 m in length (Figure 4).

A total of 9 pits were filled with substrates and arranged in a completely randomized block design. Each experimental unit was covered with a mosquito net featuring a 2 mm square mesh to prevent earthworms from escaping and to exclude other macroinvertebrates from entering.

Indigenous earthworms (*Eudrilus eugeniae*) and exotic earthworms (*Eisenia foetida*) were introduced at a density of 3 individuals per unit, based on their weight. The pits were filled with substrate, either containing or lacking earthworms, arranged in a completely randomized block design with 9 treatments, each replicated 3 times.

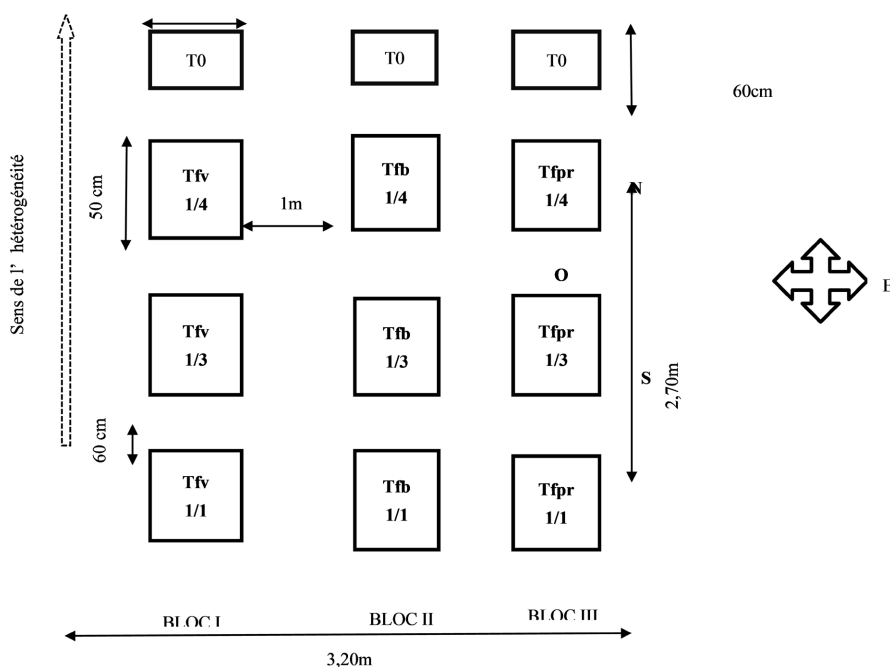


Figure 4. Experimental block design in a natural environment.

3.4.2. Tests Conducted under Controlled Conditions in Vermiculture

The controlled-condition vermiculture test aims to optimize the decomposition of organic matter using epigeic worms. This study also measures the degradation rate, worm reproduction rate, and the quality of the compost produced. The research was conducted in a controlled environment utilizing ani-

mal waste, municipal waste, and green waste at varying incorporation rates. The objective was to determine the pre-compost/soil ratio that is most favorable for earthworm survival and to assess the effect of earthworms on the substrate.

Setting up and monitoring the experiments:

The experiments were carried out in plastic bins (45 at each site) arranged horizontally as compost bins. Each bin had dimensions of $45 \times 45 \times 20$ cm and contained three earthworms. To maintain moisture for the worms, the bins were covered with straw. Following Chaoui's method [11], the total duration of the experiment was 40 days. This period included not only the active decomposition phase (spanning from days 22 to 32) but also a necessary maturation period for the organic waste to transform into compost. In this experiment, two types of waste were utilized: plant waste and municipal waste, which were prepared into the following substrate types: (i) T Du: municipal waste (100%) (ii) T fv: manure poultry (5%) + plant waste (95%) (iii) T v + u: plant waste (2/3) + municipal waste (1/3) (iv) T u + v: plant waste (1/3) + municipal waste (2/3) (v) T n: bare soil.

Experimental design: The study tested two categories of earthworms (native and exotic), five types of substrates, and conducted three replicates for each substrate, along with a control (Figure 5). The fifteen treatments were arranged in a full factorial design (Figure 6).

3.4.3. Method for Identifying Earthworms

The two categories of earthworms were identified at the Laboratoire d'Entomologie et de Parasitologie at the Faculté des Sciences et Technique of the Université des Sciences Techniques et Technologies de Bamako.

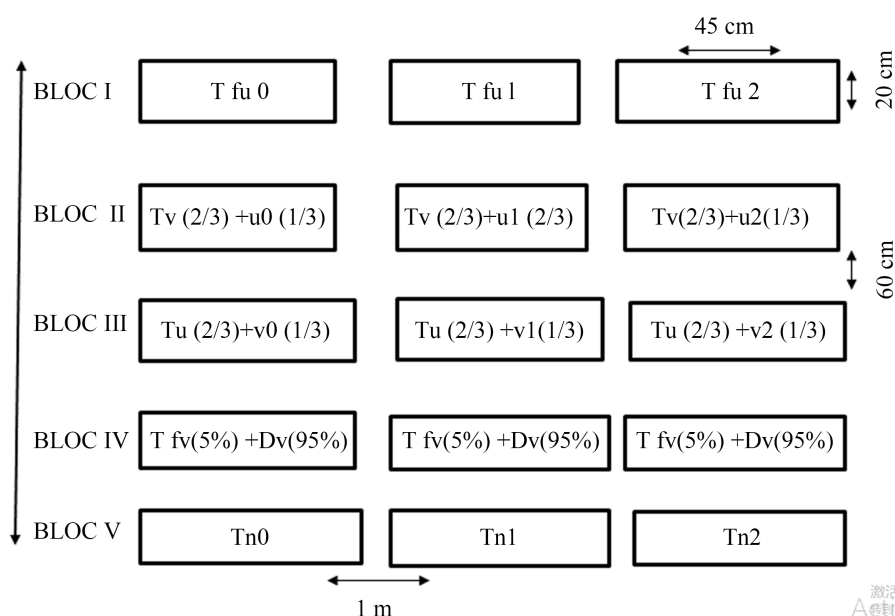


Figure 5. Block design in a controlled environment.



Installation of the brackets



Weighing waste



Mixing proportions

Figure 6. Installation of experimental setups in a controlled environment.

Measuring length (height)



Weight gain



Observation under a binocular magnifying glass

Figure 7. Different stages of earthworm identification.

The collected earthworms were identified using the identification key from [8].

A total of twenty samples were selected, consisting of ten individuals of *Eisenia foetida* and ten of *Eudrilus eugeniae*. The worms were rinsed with water in a Petri dish before being fixed in alcohol to immobilize them. This step facilitated the observation of various morphological characteristics under a binocular magnifying glass, including color, number of segments, arrangement of setae, shape of the prostomium, and shape of the tail. Weight and size were measured using a precision balance and a graduated ruler, respectively (Figure 7).

4. Results

4.1. Viability of Worms in Different Substrates under Natural Conditions

Figure 8 shows that the observed viability rate was 100% for the proportions FPR-1/1, 1/3, and 1/4. A 67% viability rate was noted for the FPR-1/0 proportion.

4.2. Viability of Worms in Poultry Manure Substrates + Soil

The results presented in Figure 9 indicate that the FV-1/1 group had a viability rate of 0%, resulting in three recorded deaths. In contrast, the FV-1/3 group exhibited a viability rate of 67%, with one death recorded. The FV-1/4 group achieved a 100% viability rate, with no deaths reported. Similarly, the FV-1/0 group also showed a viability rate of 67%, with one death recorded.

4.3. Viability of Worms in Cattle Manure Substrates + Soil

Figure 10 displays the following viability rates: 67% for the FB-1/1 ratio (indicating recorded mortality), 100% for the FB-1/3 ratio (indicating no mortality), 100% for the FB-1/4 ratio, and 67% for the FB-1/0 ratio.

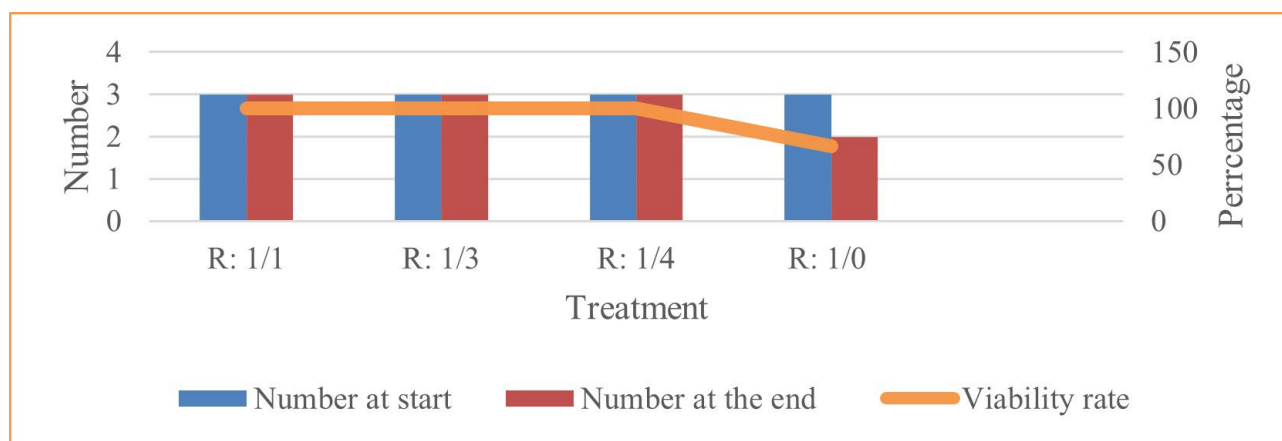


Figure 8. Viability of worms according to the proportion rate of FPR substrates + soil.

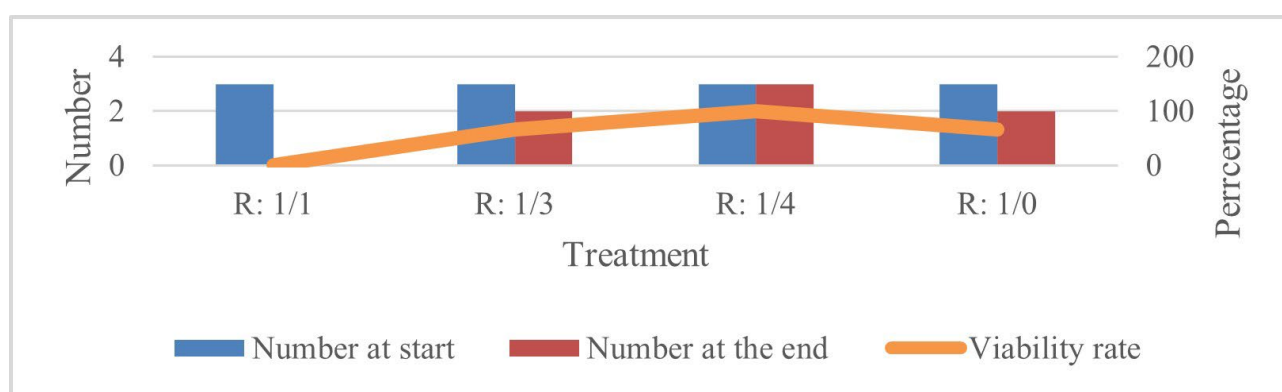


Figure 9. Viability of worms according to the proportion rate of poultry manure substrates + soil.

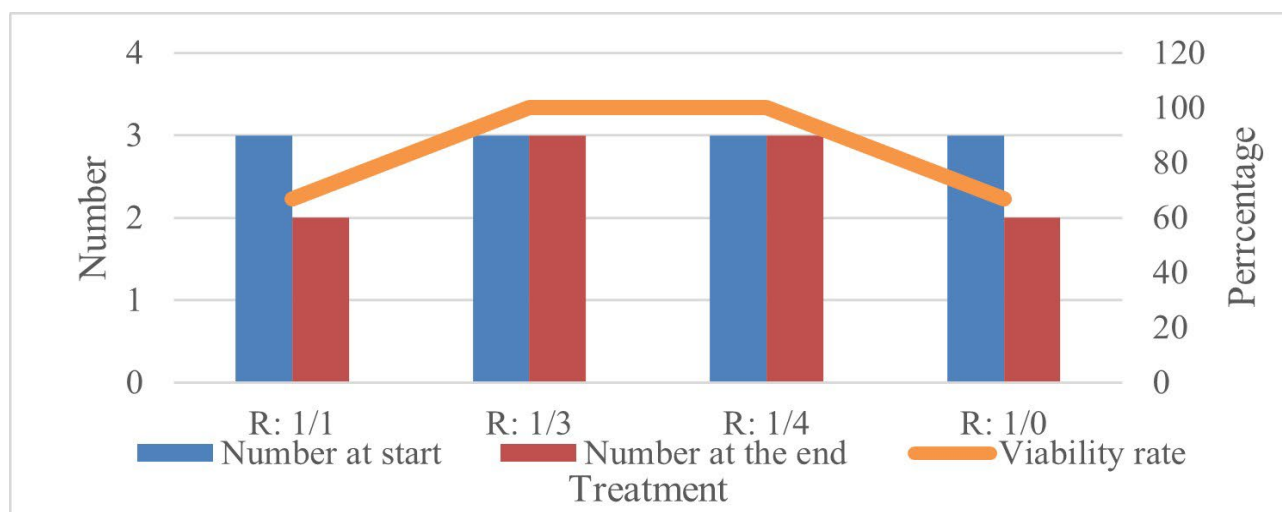


Figure 10. Viability of worms according to the proportion rate of cattle manure substrates + soil.

4.4. Viability of Worms in Different Substrates under Controlled Conditions

Figure 11 indicates a 100% survival rate for the species *E. eugeniae* and *E. foetida* when treated in urban waste.

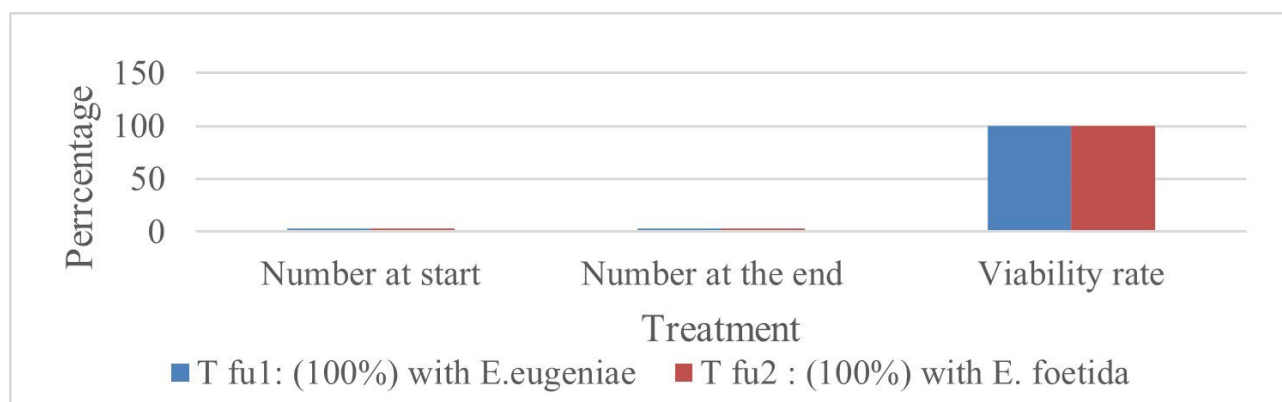


Figure 11. Worm viability in the urban manure (100%).

An analysis of **Figure 12** indicates a 100% viability rate for both categories of earthworms in the mixture of plant waste (2/3) and municipal waste.

Figure 13 presents a survival rate of 100% for *Eudrilus eugeniae* when using a mixture of one-third plant waste and two-thirds municipal waste. In contrast, *Eisenia foetida* showed a survival rate of 67% with the same mixture of one-third plant waste and two-thirds municipal waste.

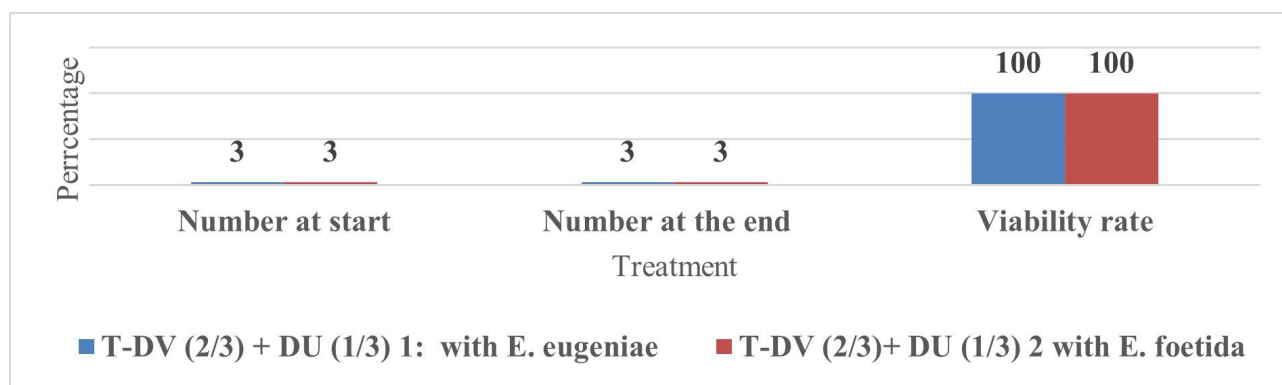


Figure 12. Worm viability in Tv (2/3) + DU (1/3).

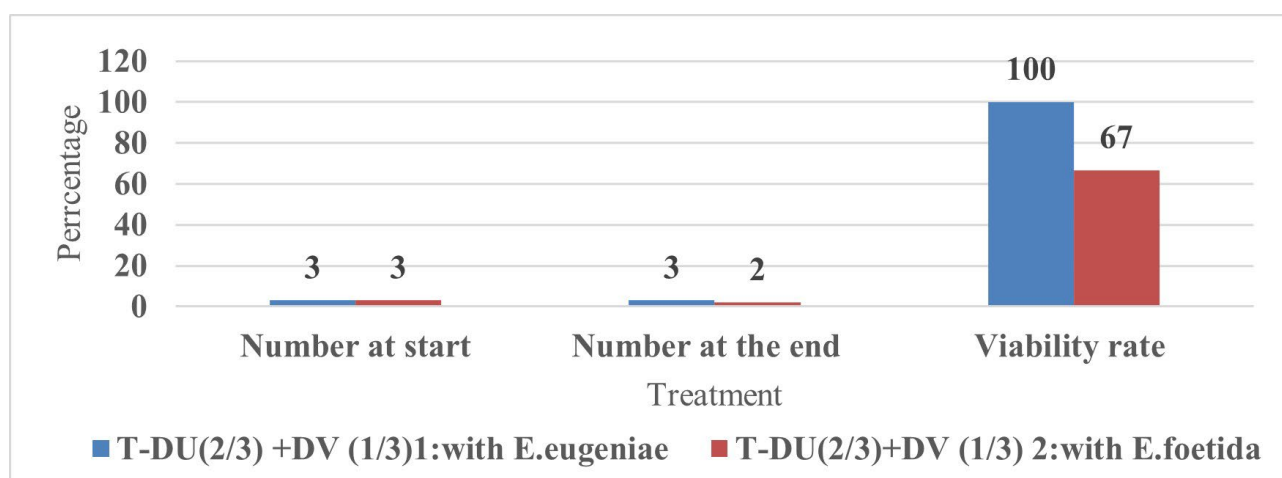


Figure 13. Worm viability in T-DU (2/3) + DV (1/3).

Figure 14 illustrates a viability rate of 67% for the species *Eudrilus eugeniae* and *Eisenia foetida* when using a mixture of 95% plant waste and 5% poultry manure.

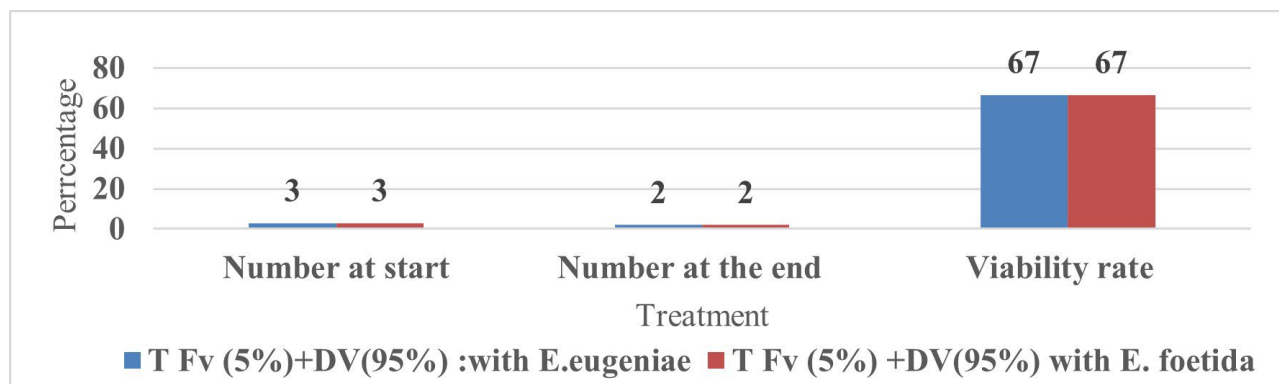


Figure 14. Worm viability in the DV (95%) + FV (5%).

4.5. Viability of Worms in the Bare Soil Test Substrates

Figure 15 displayed a mortality rate of 100%, which corresponds to 3 deaths, for the species *Eisenia foetida*. In contrast, the survival rate for the species *Eudrilus eugeniae* was 67%, indicating one death.

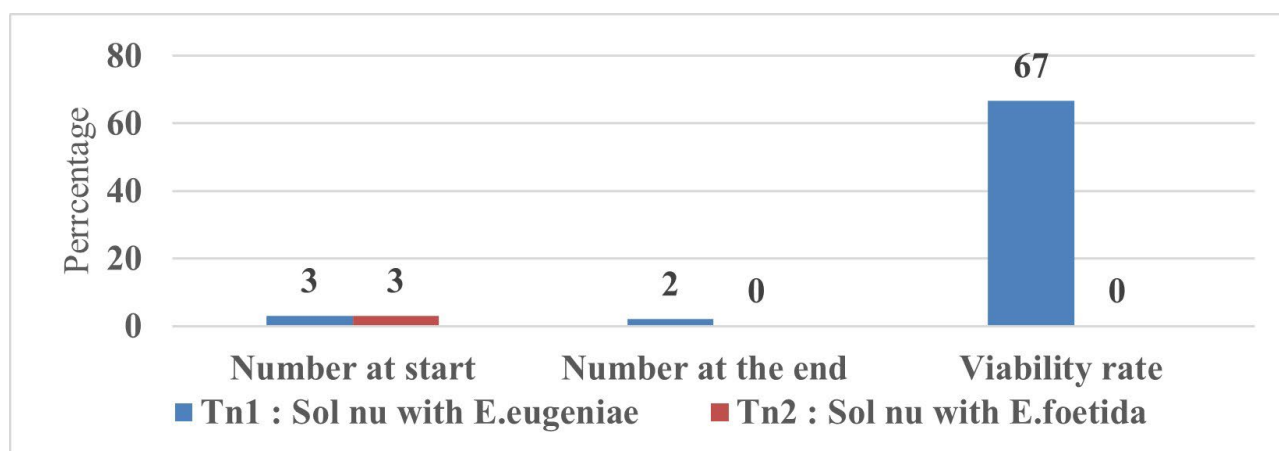


Figure 15. Viability of worms in bare soil.

4.6. Identification and Characterization of Earthworm Species

In this study, two species of earthworms were identified and described using the identification keys from [8] (**Table 2**), (**Figure 16**), (**Figure 17**).

Table 2. Comparison of the morphological characteristics of the different collected earthworm species.

Morphological characteristics	Species	
	<i>Eisenia foetida</i>	<i>Eudrilus eugeniae</i>
Weight (g)	0.79	2.33
Length (size) cm	9.26	16.68

Continued

Number of segments	158.7	267
Clitellum	Saddle	Saddle
Color	Red	Black
Setae	Distant pair	Close pair
Prostomium shape	Epilobous	Zygotobous
Clitellum shape	Saddle	Saddle
Tail shape	Flattened	Circular
Species type	Epigeic	Anecic



The average weight of a worm is 0.79 g;
The average length is 9.26 cm;
The average number of segments is 159;
The clitellum is saddle;
The worms are red in color;
The setae are distant pair;
The tail shape is flattened;
The prostomium is epilobous.

Figure 16. Identification of the species *Eisenia foetida*.



The average weight of a worm is 2.33 g;
The average length is 16.68 cm;
The average number of segments is 267;
The clitellum is saddle;
The worms are black in color;
The setae are in close pairs;
The tail shape is circular;
The prostomium is zygotobate.

Figure 17. Identification of the species *Eudrilus eugeniae*.

5. Discussion

The zootechnical importance of earthworms:

Earthworm production in livestock farming is a sustainable and cost-effective alternative for poultry feed. Expanding the use of earthworms in livestock farming necessitates a deeper understanding of the subject to optimize both earthworm production and vermicomposting.

5.1. Identification and Characterization of Earthworm Species

Earthworms play a crucial role in the dynamics of soil nutrients by changing the physical, chemical, and biological properties of the soil. They consume decom-

posing organic matter and soil mixed with organic materials, which enhances soil fertility, recycles organic matter, and enriches the soil with nutrients through their castings.

5.1.1. Morphological Characteristics of the Species *Eisenia foetida*

The average size of *E. foetida* recorded in this study is 9.26 cm, which is comparable to the values reported by [12] at 9 cm and [13] at 8.5 cm. It exceeds the value reported by [14], which was 8 cm. The study also found an average weight of 0.79 g, higher than the weights reported by [7] and [15], which were 0.699 g, and [16], which was 0.078 g.

In terms of coloration, the observed red hue aligns with findings from [7], who described it as purplish-red with stripes and dark red, respectively. However, [13] reported brown coloration, while [12] noted yellow-striped patterns.

Additionally, the study revealed that the prostomium type is Epilobic, consistent with observations by [13] and [14], who also reported the same Epilobic shape. In contrast, [7] described the prostomium as zygotilobic.

The investigation further indicated that the bristle position observed was of the Distant Pair type. This contrasts with findings from [12] and [14], who reported Germinate and Separate bristle positions, respectively.

The average number of segments noted was 159, which is higher than the counts documented by [13] and [14], at 90 and 101 segments, respectively. However, it is slightly lower than the 162 segments reported by [7].

The tail shape observed was flattened, similar to the cylindrical shape with slight caudal flattening reported by [14] and the cylindrical shape with flattening noted by [13].

Finally, the study identified the clitellum shape as saddle-shaped, which does not align with the annular shape observed by [7].

5.1.2. Morphological Characteristics of the Species *Eudrilus eugeniae*

The average number of segments obtained was 267. This value is higher than those reported by [12]-[15], which were 207, 175, and 207 segments, respectively.

The average size observed was 16.68 cm. This result corroborates that reported by [17], which is 17 cm. It is lower than that reported by [16], which is 11.2 cm. It is higher than those obtained by [7] and [12], who obtained 15 cm.

The shape of the clitellum observed was saddle-shaped. This result contrasts with that obtained by [7], which was annular.

The position of the setae observed was close-set. However, this is contrary to the findings reported by [7] and [12], who found the setae to be separated.

The prostomium shape is zygotilobic. This result contradicts that obtained by [7], which is open epilobic.

The observed coloration is black. This result does not corroborate those observed by [7]-[16], who obtained dark pink to violet and dark red, respectively.

The average weight obtained was 2.33 g. This result is similar to that obtained by [17], which is 2 g, and is lower than that obtained by [7], which is 4 g.

The shape of the tail observed was circular.

6. Conclusions

This study highlights that vermiculture is emerging as a strategic and sustainable solution for animal production. Additionally, soil-dwelling earthworms play a crucial role in enhancing soil fertility, improving drainage, and recycling organic matter in tropical agrosystems. A deeper understanding of their biology and ecology is essential for harnessing their benefits in agricultural, agrosilvopastoral, and economic productivity, particularly in the Sudano-Sahelian zone. By synthesizing the existing knowledge on earthworms, we can facilitate their application in improving livestock productivity.

Based on the findings, the Badougou Djoliba area in Mali, which is characterized by a Sudano-Sahelian climate, presents significant potential for earthworm farming due to the availability of raw materials. Considering all factors, utilizing earthworms as a protein source could provide both zootechnical and economic advantages for animal production, with further development opportunities that remain to be explored through additional studies.

Conflicts of Interest

The authors declare no conflicts of interest.

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Abbreviations and Acronyms

Fpr (small ruminant manure),
 Fv (poultry manure),
 Fb (cattle manure),
 Sol nu (soil),
 DU (urban waste),
 DV (plant waste),
 T (test).