Original Research Article

Growth and reproduction performance of the earthworm (*Eudriluseugeniae*) fed with different substrates made of palm oil empty fruit bunches: A case of vermicompost production (Abidjan, Côte d'Ivoire)

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ABSTRACT

The objective of the present study is to analyze the growth capacity and activity of the worm species *Eudriluseugeniae* in different substrates based on palm oil empty fruit bunches as part of the production of vermicompost. To this end, 4 treatments were carried out by adding to palm oil empty fruit bunches other substrates readily available in the environment, notably *Chromolaena odorata* leaves and grass clippings. 1.5 L plastic boxes were filled with 800 g of soil and 300 g of various precomposted substrates. In each box, 4 earthworms (*Eudriluseugeniae*) were seeded. A total of 4 treatments were carried out : T1 (palm oil empty fruit bunches + earthworms), T2 (palm oil empty fruit bunches + *Chromolaena odorata* + earthworms), T3 (palm oil empty fruit bunches + grass clippings + earthworms) and T4 (palm oil empty fruit bunches + *Chromolaena odorata* + grass clippings + earthworms). For the different vermicomposts based on palm oil empty fruit bunches, growth parameters and *Eudriluseugeniae* activity were better in treatments T3 and T4. These same treatments also showed significant variation in substrate quantity after vermicomposting. The combination of palm oil empty fruit bunches and green waste (*Chromolaena odorata* and grass clippings) would be ideal for the production of vermicompost with good agronomic potential to be applied as an organic amendment to cultivated plants.

Key words :Eudriluseugeniae, Survival, palm oil empty fruit bunches, Co-composting, Green waste.

1. INTRODUCTION

Soils are teeming with numerous living organisms [1]. Among these organisms, earthworms play an important role in their maintenance and survival, as well as in the decomposition of organic matter [2]. These organisms are involved in the decomposition of the soil's organic matter complex, helping to release the mineral elements needed by plants. Their activity thus affects plant growth and development, and the spatial distribution of nutrients in the soil [3]. Earthworms are also considered “ecosystem engineers”, as they play an important role in creating and maintaining soil structure [4]. They also provide a number of ecosystem services [5]. Despite the vast amount of knowledge accumulated about these organisms, and the formal recognition of their importance to soil, ecosystem and society, many new and more important discoveries remain to be made. For example, behavioral and physiological aspects continue to interest scientists, as many questions remain unanswered for many earthworm species [3-6]. These organisms consume virtually all types of decomposing organic matter mixed with soil (crop residues, bacteria, algae, protozoa, fungi, nematodes, rotifers) [2]. Therefore, by means of vermicomposting, earthworms could be an ideal solution to the problem of long-term agricultural and agro-industrial waste management, to the enhancement of organic farming through the substitution of organic fertilizers to chemical fertilizers. However, their contribution to the decomposition of organic matter, as well as their growth, survival and reproduction, are often influenced by environmental climatic factors and the chemical composition (C/N, lignin, polyphenolic compounds, etc.) of the organic matter [7]. Several studies have therefore been carried out on earthworm population dynamics, using substrates of various natures. These have revealed that their food preference and growth vary according to species and substrates [8-9]. Moreover, in the case of palm oil fruit bunches, liquid waste (liquid sludge, washing water, etc.) and solid waste (empty fruit bunches) are generally discharged untreated into the environment [10]. This causes major management challenges in many tropical countries in general, and developing countries in particular [11]. However, this waste combined with other easily accessible substrates can be used in the vermicomposting process, which is a new, reliable alternative for increasing agricultural production and restoring soil productivity [12]. It is in this context that the present study was conducted, in order to monitor the growth potential of the earthworm species *Eudriluseugeniae*, which is a manure worm much used for vermicompost production in tropical Africa [13]. To this end, (i) the demographic parameters of *Eudriluseugeniae* worms were monitored in the various substrates formulated with palm oil empty fruit bunches, (ii) the growth parameters of the worms were determined and (iii) the variation in the quantity of residues under the action of earthworms was examined.

2. MATERIAL AND METHODS

2.1. Study area

The work was carried out in the autonomous district of Abidjan at the Nangui ABROUGOUA University, located between latitudes 5°21 and 5°23 north and longitudes 4°01 and 4°09 west. This university lies midway between the communes of Adjamé and Abobo. It is bounded to the north by the commune of Abobo, to the south by Adjamé-Williamsville, to the east by the HôpitalMilitaired'Abidjan (HMA) and to the west by the Adjamé-Abobo road and Filtisac (spinning mill). The climate is humid sub-equatorial.

2.2. Substrate preparation and precomposting

The substrates used for the experiment were oil palm empty fruit bunches from an oil refinery of Aboisso department, Chromolaena odorata leaves from various fallows near the city of Abidjan, and grass clippings from the univesity. These were first dried at room temperature for a week (Fig 1), then finely ground in a mortar and sieved with a 2 mm mesh sieve. Secondly, they were precomposted for 6 weeks to facilitate worm activity and digestion of the organic matter in their digestive tracts.

Fig1: Organic substrates dried at room temperature (A. Oil palm empty fruit bunches; **B.** Chromolaena odorata leaves; **C.** Grass clippings**)**

2.3. Collection of earthworms and soil

Sub-adult earthworms of the *Eudriluseugeniae* with masses ranging from 0.8 g to 1.03 g were collected from windrows (10 m x 1 m) set up within the university (Fig 2). A total of 400 earthworms were collected for the experiment. In addition, soil samples were taken from the same worm collection site and added to the various substrates in order to monitor the demographic parameters of the earthworms. These soils were first air-dried for a week on a support in the shade, then sieved with a 2 mm mesh sieve to eliminate coarse elements and facilitate the movement of the earthworms in the different compost bins (Fig 3).

Fig 2: Worm trapping windrows

Fig3: Shade-dried soil

2.4. Experimental design

The compost bins consisted of 100 plastics boxes (1.5 L), with perforated closures for better aeration (Fig 4). The bottom of each box was pierced with tiny holes to allow water draining to avoid excessive water in the boxes [15]. Each box was firstly filled with 200 g of lightly hydrated soil to provide shelter for the earthworms. Then, 800 g of the various precomposted substrates were added and 4 earthworms were addedaccording to [16]. Each treatment was repeated 25 times. Each of these treatments had the following composition:

- **T1 :** Oil palm empty fruit bunches + Earthworms;
- **T2 :** Oil palm empty fruit bunches + *Chromolaena odorata* + Earthworms;
- **T3 :** Oil palm empty fruit bunches + Grass clippings + Earthworms;

- **T4:** Oil palm empty fruit bunches + *Chromolaena odorata* + Grass clippings + Earthworms.

Fig4: Composter made of 1.5 L plastic boxes

2.5. Determination of earthworm demographic and growth parameters

The demographic and growth parameters of the earthworms were determined after 60 daysof experiment. Adult worms, juveniles and cocoons were collected, counted and weighed.

2.5.1.Survival rate

The survival rate was determined by taking into account the total number of earthworms used at the beginning of the experiment and the total number of earthworms obtained at the end of the experiment, according to the following formula :

$$\mathbf{SR} = \frac{\mathbf{N}i}{Nf} \times \mathbf{100}$$

where: **SR:** survival rate; **Ni:** number of earthworms at the beginning of the experiment; **Nf:** number of earthworms at the end of the experiment.

2.5.2. Birth rate

The birth rate was determined by taking into account the number of juvenile worms and the size of the population, using the following formula :

$$\mathbf{BR} = \frac{\mathbf{Nj}}{Na} \times \mathbf{100}$$

where :**BR**: birth rate; **Nj**: number of juveniles ; **Na**: population size (adults + juveniles).

2.5.3.Weight gain

The average weight gain, used to evaluate the weight growth of the worms during a given period, was determined using the formula in [17] :

$$\mathbf{AWG} = \mathbf{W}\boldsymbol{f} - \mathbf{W}\boldsymbol{i}$$

where, **AWG**: average weight gain (g), **W*f***: average weight of worms (g) at the end of the experiment (tf), **W*i***: average weight (g) of worms at the beginning of the experiment (ti).

2.5.3. Relative growth rate

Relative Growth Rate (RGR) is the growth relative to population size or individual mass. RGR, which is a measure used to quantify the growth rate of an organism, corresponds to the increase in mass per day, relative to the initial biomass. It's expressed in $g^{-1}\ d^{-1}$. RGR was determined according to the formula following formula [16]:

$$\mathbf{RGR} = \frac{\mathbf{AWG}}{\boldsymbol{Wi}} \times \mathbf{100}$$

where, **RGR :** Relative growth rate; **Gmp :** Average weight gain (g), **Pi :** Initial worm weight (g).

2.5.4. Determination of the percentage loss of substrate quantity

The percent loss of substrate reduction was determined by the following formula :

$$\mathbf{PSR} = \frac{\boldsymbol{WSi} - \boldsymbol{WSf}}{\boldsymbol{WSi}} \times \mathbf{100}$$

where, **PSR:** Percentage of substrate reduction; **WSi:** Substrate weight before the start of rearing; **WSf:** Substrate weight at the end of the rearing period.

2.5.5. Statistical analysis

Data were analyzed using R 3.2.1 software. One-way analysis of variance (ANOVA1) was used to compare the means of the various parameters measured. Before performing these comparison tests, the normality of the data was checked using the ShapiroWilk test. The significance threshold of the probability value for each test was set at 0.05. Turkey's HSD post-hoc test was used to test the significance of the differences.

3. RESULTS AND DISCUSSION

3.1. Variation of earthworm growth parameters (Survival rate, Birth rate, Average weight gain and Relative growth rate)

After 6 weeks of experiments, of the 400 earthworms seeded, 326 survived and a total of 3582 juveniles were obtained. Results of survival and birth rates determined for the 4 types of treatment, are shown in Table 1. For both survival and birth rates, the highest values were recorded in treatments T4 (25.30±23.33% and 93.74±1.46% respectively), T3 (22.47±8.78% and 92.72±1.16% respectively), which are statistically identical. On the other hand, values for these different rates were lower in treatments T2 (7.43±1.21% and 85.05±7.07% respectively) and T1 (6.73±1.45% and 82.66±17.97% respectively), which were also statistically identical. In all cases, the survival and birth rates in treatments T4 and T3 were both significantly different (p<0.05) from those in treatments T2 and T1, between which there was no significant difference (P>.05).

Table 1 : Survival and birth rates of *Eudriluseugeniae* in the different treatments

Type of treatment	*SR* (%)	*BR* (%)
T1	6.73±1.45 ^{b}	82.66±17.97 ^{b}
T2	7.43±1.21 ^{b}	85.05±7.07 ^{b}
T3	22.47±8.78 ^{a}	92.72±1.16 ^{a}
T4	25.30±23.33 ^{a}	93.74±1.46 ^{a}

SR: survival rate; BR: birth rate; **T1:** Oil palm empty fruit bunches + earthworms; **T2:** Oil palm empty fruit bunches + Chromolaena odorata + earthworms; **T3:** Oil palm empty fruit bunches + grass clippings + earthworms; **T4:** Oil palm empty fruit bunches + Chromolaena odorata + grass clippings + earthworms.
Values followed by the same letter in a column are not significantly different (p>.05) according to the tukey test.

For both average weight gain (AWG) and relative growth rate (RGR), the highest values were observed in treatment T4 (0.90±0.02 g and 98.80±5.77% respectively) followed by treatment T3 (0.07±0.01 g and 78.94±7.66% respectively). Treatment T1, on the other hand, had the lowest values (0.90±0.03 g and 22.22±4.57% respectively). For all treatments (T1, T2, T3 and T4), significant differences were observed between each (p<.05) (Table 2).

In a vermicomposting system, substrate quality is an important factor for earthworm development and growth [18]. Thus, for the different vermicomposts made of oil palm empty fruit bunches, the growth parameters (survival rate, birth rate, average weight gain and relative growth rate) and activity (capacity to decompose substrates) of *Eudriluseugeniae* were better in treatments T3 (Oil palm empty fruit bunches + Grass clippings) and T4 (Oil palm empty fruit bunches + *Chromolaena odorata* + Grass clippings). These results indicate the possibility of obtaining high-quality vermicompost from the co-composting of Oil palm empty fruit bunches not only with grass clippings (Poaceae), but also with the combination of different types of plant residues, in particular *Chromolaena odorata* + grass clippings. These results corroborate those of [19], who showed that the proportion and quality of substrates used in a treatment influence both the mineral content of the compost and the growth and reproduction of earthworms. While Oil palm empty fruit bunches alone had the potential to improve the growth parameters of *Eudriluseugeniae*worms, the addition of grass clippings effectively boosted their performance. This was not the case with the addition of *Chromolaena odorata* leaves, which made a lower contribution to performance enhancement. This difference may be due to the fact that the proportion of nutrients favourable to the growth of *Eudriluseugeniae* is greater in the grass clippings than in the *Chromolaena odorata* leaves. Numerous authors have demonstrated that good growth of *Eudriluseugeniae* in a substrate is linked to the nature and quantity of the mineral elements in that substrate, particularly nitrogen (N) and the ratio of carbon to nitrogen (C/N) [20,21]. According to [21], the proportions of these minerals in grass clippings appear to be so high that this substrate is considered excellent for the production of high-quality vermicompost.

However, the simultaneous addition of *Chromolaena odorata* leaves and grass clippings to oil palm empty fruit bunches very effectively improves the growth parameters of *Eudriluseugeniae*. This is considerably better than adding grass clippings alone. On the other hand, their effect is significantly higher than that of the combination of oil palm empty fruit bunches and *Chromolaena odorata* leaves, and also than that of oil palm empty fruit bunches. Studies carried out by [20] on the use of three agricultural by-products (cocoa bean husk, coffee parchment and sawdust) for the production of vermicompost have shown that the combination of several substrates makes it possible to make the most of their beneficial contributions and minimize any drawbacks linked to their respective chemical compositions.

Table 2 : Weight gain and relative growth rate of *Eudriluseugeniae* in the different treatments

Type of treatment	AWG (g)	RGR (%)
T1	0,20±0,03 ^{d}	22,22±4,57 ^{d}
T2	0,41±0,04 ^{c}	44,32±4,75 ^{c}
T3	0,70±0,01 ^{b}	78,94±7,66 ^{b}
T4	0,90±0,02 ^{a}	98,80±5,77 ^{a}

AWG: Average weight gain; **RGR:** Relative growth rate; **T1:** Oil palm empty fruit bunches + earthworms; **T2:** Oil palm empty fruit bunches + *Chromolaena odorata* + earthworms; **T3:** Oil palm empty fruit bunches + grass clippings + earthworms; **T4:** Oil palm empty fruit bunches + *Chromolaena odorata* + grass clippings + earthworms.
Values followed by the same letter in a column are not significantly different (p > 0.05) according to the tukey test.

3.2. Variation in residue quantity

After 6 weeks of earthworms monitoring, the highest percentage substrate loss was observed in treatment T4 (37.90±97.57%), followed by treatments T3 (35.92±97.90%), T2 (21.88±98.18%) and T1 (14.02±97.94%). However, statistical analyses revealed that the substrate loss rates of treatments T4 and T3 were both significantly different (p<.05) from those of treatments T2 and T1, which did not show any significant difference between them (P>.05) (Table 3).

Furthermore, among the different treatments analyzed, the significant variation in substrate quantity observed in treatments T3 and T4 after vermicomposting could express their great capacity to be mineralized under the influence of the activity of *Eudriluseugeniae* manure worms. In addition, these earthworms would have been actively involved in decomposing the organic matter present and releasing plant-available nutrients. These results match those of [23]who, in their studies on the manure worm *Eudriluseugeniae*, demonstrated that when environmental conditions are favorable for earthworms, they have the capacity to facilitate the mineralization of the organic matter at their disposal. In addition, the significantly higher mineral content of vermicomposts from treatments T3 and T4 is linked to the activity of the microbial flora present in the earthworms' digestive tract, which is involved in the mineralization process. Our results are in agreement with those of [24], who used vermicomposting of municipal organic waste to demonstrate the effectiveness of earthworms in ensuring efficient mineralization of the substrates at their disposal.

Table 3 : Variation in substrate quantity for different types of treatment

Type of treatments	Initial dry matter (g)	Final dry matter (g)	PSR(%)
T1	300	257,92±2,06	14,02±97,94 ^{b}
T2	300	234,34±1,82	21,88±98,18 ^{b}
T3	300	192,05±2,10	35,92±97,90 ^{a}
T4	300	186,28±2,43	37,90±97,57 ^{a}

PSR : Percentage of substrate reduction; **T1 :** Oil palm empty fruit bunches + earthworms; **T2 :** Oil palm empty fruit bunches + *Chromolaena odorata* + earthworms; **T3:** Oil palm empty fruit bunches + grass clippings + earthworms; **T4:** Oil palm empty fruit bunches + *Chromolaena odorata* + grass clippings + earthworms.
Values followed by the same letter in a column are not significantly different (p>.05) according to the tukey test.

4. CONCLUSION

Oil palm empty fruit bunches combined only with grass clippings or with grass clippings plus *Chromolaena odorata* lead to much better demographic parameters (survival, birth rate) and activities (weight gain and relative growth rate) for the dung worm *Eudriluseugeniae*. These same substrates from co-composting also have excellent capacities to be degraded by the action of *Eudriluseugeniae*. The combination of oil palm empty fruit bunches with easily accessible green waste, in particular grass clippings alone or grass clippings + *Chromolaena odorata* (herbaceous shrubby plant widely distributed in fallow land) seems to be ideal for obtaining vermicompost with interesting agronomic characteristics.

COMPETING INTERESTS

The authors declare no conflict of interest.

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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