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2 **Original Research Article**

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4 **COPROLITES PRODUCTION OF NATIVE EARTHWORMS IN**
5 **BRAQUIARIA FIELDS UNDER BIOFERTILIZATION**

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33 **ABSTRACT**
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Aims: The objective of this study was to evaluate the coprolite production of native earthworms in a pasture with Brachiaria, with and without liquid-enriched biofertilization.

Place and Duration of Study: The experiment was carried out between April of 2014 and August of 2015 at the Centro de Ciências Agrárias da Universidade Federal da Paraíba – UFPB.

Methodology: A randomized complete block experimental design was used, with subdivided plots and four replicates, with a total of 40 plots in a 5x3x2 factorial arrangement,

five grass species (*Brachiaria brizantha*, *B. decumbens*, *B. humidicola*, *B. ruziziensis* e *B. brizantha* MG5) and three sampling times, with and without liquid-enriched biofertilization. The plot area was composed of 50.0 m² (10.0 m x 5.0 m) with subplots of 0.25 m² (0.5 m x 0.5 m). Six foliar fertilization were performed in intervals of fifteen days, with three applications in the drought period and three applications in the rainy season. Each application consisted of 5% of biofertilizer (100 mL of biofertilizer diluted in 2 L of water), each plot received 2 L of biofertilizer.

Results: In the dry season there was a significant difference in the means between the fertilization treatments, leading to the absence of fertilization, obtaining a better result, varying of 48, 24% in relation to the treatments that received fertilization, and there was no significant difference between the brachiaria. In the rainy season, it was verified that there was no significant difference in the means between the fertilization treatments, but there was a significant difference between the brachiaria.

Conclusion: The production of earthworm coprolites was higher under pasture with *Brachiaria* MG5 in the drought season. In the rainy season, the coprolite production increased under pasture with *B. humidicola*. The application of liquid-enriched biofertilizer in *Brachiaria* promoted lower production of earthworm coprolites.

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Keywords: Organic fertilization, soil biology, soil fertility.

1. INTRODUCTION

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Earthworms are among the organisms that compose the macrofauna, these organisms are one of the most important invertebrates of ecosystems and agroecosystems around the world [1,2]. They are considered engineers of the ecosystem with a large impact on the soil structure [3]. Earthworms are estimated to be responsible for about 40% to 90% of the biomass of edaphic macrofauna in most tropical ecosystems. They participate in the incorporation and decomposition of organic matter present in the soil when they ingest the organic matter added to the inorganic matter of the soil, which passes through the intestinal tract and then is excreted as coprolites (biogenic aggregates), all these factors contribute to the soil quality improvement, since they are directly linked with particle aggregation and nutrient availability [4,5,6].

Coprolites contain calcium humate, which together with the calcium released by the calciferous glands, serves as an aggregating element to the soil particles [7]. In addition, they contain large amounts of nutrients, due to the addition of organic matter and urinary and intestinal secretions that forms a homogeneous and rich structure, the movement of organic matter and mineral components through the digestive tract of earthworms is subjected to enzymatic processes and break downs, which increases soil fertilization [8].

The insertion of earthworm coprolites in crop cultivation guarantees not only superior plant nutrition but also the substrate quality when incorporated, in addition, the low cost of this input makes its use viable [9]. Several studies have pointed out the importance of vermicomposting for agricultural production, especially with regard to the improvement of commercial crops and pastures [10, 11, 12, 13, 14]. However, it is not known how the application of biofertilizer and seasonality can affect the production of coprolites in a native pasture environment.

The objective of this study was to analyze the production of "geophagous" native earthworm coprolites in a Yellow Oxisol area, in the city of Areia-PB, under the pastures of *Brachiaria* grasses, with and without liquid-enriched biofertilizer.

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72 2. MATERIAL AND METHODS

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74 The experiment was carried out between April of 2014 and August of 2015 in the
75 experimental station "Chã do Jardim", at the Centro de Ciências Agrárias of the
76 Universidade Federal da Paraíba - UFPB, Areia - PB. The soil of the experimental area is
77 classified as Yellow Oxisol, deep, well-drained and sand-clay texture [15].

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79 A randomized complete block design was used, with subdivided plots and four replicates,
80 with a total of 40 plots (Figure 1). The factorial arrangement used was 5 x 3 x 2, with five
81 species of grasses (*Brachiaria brizantha*, *B. decumbens*, *B. humidicola*, *B. rriziensis* *B.*
82 *brizantha* MG5), three sampling times in with and without liquid-enriched biofertilization.

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85 **Fig.1. Experimental area: arrangement of plots and subplots for application of**
86 **treatments with and without organic fertilizer, Chã do Jardim, Areia - PB.**

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88 The plot area had 50.0 m² (10.0 m x 5.0 m) with subplots of 0.25 m² (0.5 m x 0.5 m). Six
89 biweekly applications via foliar fertilizations were performed, with three applications in the
90 drought season and three applications in the rainy season. Each application consisted of 5%
91 of biofertilizer (100 mL of biofertilizer diluted in 2L of water), each plot received 2 L of
92 biofertilizer).

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94 Coprolites produced by native "geophagous" earthworms were manually collected over a
95 period of eight months, in a 45-day time span, between October of 2014 and January of
96 2015 (drought period) and from May to August of 2015 (rainy season). Six collections were
97 carried out in the experimental area. For the collection, an iron square was randomly placed
98 in the plots, three replications per subplot were used. The collected material was placed in
99 properly labeled containers and sent for chemical analysis in the Laboratório de Solos do
100 Centro de Ciências Humanas, Sociais e Agrárias da Universidade Federal da Paraíba,
101 Bananeiras- PB, following the methodological procedures [15].

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104 **Fig. 2. Collection of biogenic aggregates (native earthworm coprolites), using a iron**
105 **square in areas under grass pasture of Brachiaria genus, Chã do Jardim, Areia-PB.**
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107 Data were submitted to analysis of variance, using the ASSISTAT 7.7 software and means
108 compared by the Tukey test at 5% probability [16].
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110 111 **3. RESULTS AND DISCUSSION**

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113 Significant differences were observed between the fertilization treatments in the first
114 sampling (Table 1), leading to better results in the treatments with no fertilization, which
115 varied from 48, 24% compared to the treatments with fertilization, with no difference
116 observed between the Brachiaria species. It was verified that in this case, the production of
117 biogenic structures (coprolites) was higher without the application of the biofertilizer.
118 Regarding the five Brachiaria under the presence and absence of fertilization, B. MG5 varied
119 with 28.3% when compared to the production of B. Ruzizienses. This can be explained by
120 the fact that B. MG5 has higher production of dry matter, drought resistance, rapid regrowth
121 after grazing and better tolerance to poorly drained soils when compared to the other
122 species. In the second and third sampling (Table 1), there were no significant differences
123 between the fertilization treatments, nor between the Brachiaria species.
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125 During the sampling time, the drought season had higher air temperature and lower soil
126 moisture which contributed to the low density of the number of organisms, and,
127 consequently, reduced the coprolites production. In this sense, [12], found that there are
128 correlations between earthworm activity and abiotic factors such as humidity, light, and
129 temperature, which, in many cases, shape the supply of these ecosystem services played by
130 these organisms. [17] reported that seasonal variation associated with habitat played
131 important roles in the distribution and abundance of various earthworm species, where
132 seasonal parameters such as rainfall, relative humidity, air temperature, soil temperature,
133 and solar radiation influenced on fluctuations in population densities. [18] observed that in
134 the dry season the soil is more resistant to the deformations caused by the movement of
135 earthworms since a costly effort would be necessary to move through the soil profile. In a
136 study on earthworms in temperate areas, [19], points out that along an altitudinal gradient,
137 the climate can act as a barrier to the distribution of earthworms, and its abundance occurs
138 in a significant way through soil fertility and pasture quality.
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Table 1. Coprolites production of native earthworms under Brachiaria pastures during the drought season.

Brachiaria	1 st Sampling		Means
	Fertilized	Non-fertilized	
	-----t ha ⁻¹ -----		
Decumbens	1.06	1.47	1.26 a
Brizantha	1.18	1.51	1.35 a
Humidicola	0.96	1.65	1.30 a
MG5	1.26	1.94	1.60 a
Ruzizienses	1.22	1.88	1.55 a
Mean	1.14 B	1.69 A	-
	2 nd Sampling		
Decumbens	0.75	1.00	0.87 a
Brizantha	1.15	0.98	1.06 a
Humidicola	0.94	1.11	1.02 a
MG5	1.37	1.33	1.35 a
Ruzizienses	0.89	1.06	0.97 a
Mean	1.02 A	1.09 A	-
	3 rd Sampling		
Decumbens	0.30	0.44	0.37 a
Brizantha	0.58	0.51	0.55 a
Humidicola	0.43	0.54	0.48 a
MG5	0.55	0.49	0.52 a
Ruzizienses	0.50	0.47	0.48 a
Mean	0.47 A	0.49 A	-

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As observed in Table 2, there was an increase in coprolite production during the rainy season when compared to the drought season due to higher humidity and lower temperatures, which provides better soil moisture and creates favorable conditions for the activities in the form of biogenic aggregates. The difference between the two collection seasons is due to the ease movement capacity that the earthworm has inside the soil, provided by the moisture content of the soil in the rainy season, thus facilitating the feeding, constituted of organic compounds. According to [20], some earthworm species such as *A. dusei* (endogeic), reach the soil surface only after significant rainfall.

In the second sampling (Table 2), no significant differences were observed between the fertilization treatments, but significant differences between the Brachiaria species were observed. The coprolite production in *B. humidiculus* increased to 3.32 t / ha⁻¹, 50.90% higher when compared to *B. decumbens* with 2.20 t / ha⁻¹ of coprolite production. However, there were no significant differences between *B. decumbens*, MG5, *B. humidicola* and *B. ruziziensis*. In the third sampling, the coprolites production was not influenced by the treatments with fertilization, significant differences between the Brachiarias were observed, with higher coprolites production in pasture with *B. brizantha* (2.62 t / ha⁻¹), which did not differ from *B. decumbens*, *B. humidicola* and *B. ruzizienses*. Brachiaria has higher root biomass, a root system that aggregates the soil particles and provides conditions for the development of earthworms, which work in the soil and excrete in the form of coprolites. [21] emphasized the importance of earthworms in the growth of grasses such as maize, according to the authors the presence of earthworms of the genus *Chibui bari* favored the growth in stem diameter and increased the shoot and total dry matter of the plant, in addition, it was observed that the coprolites were sufficient for the supply of N to plants at levels equivalent to those of NPK amounts. According to [22], the addition of earthworm coprolites to dystrophic soils increased the growth of cabbage plants, especially in

169 concentrations higher than 70% of the volumetric composition of the substrate. This
 170 phenomenon did not occur in the present study, where the production of earthworm
 171 coprolites did not depend on the addition of biofertilizer, the soil itself was able to supply the
 172 nutrients necessary for coprolites production.
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174 **Table 2. Coprolites production of native earthworms under Brachiaria pastures**
 175 **during the rainy season.**

Brachiaria	1 st Sampling		Means
	Fertilized	Non-fertilized	
	-----t ha ⁻¹ -----		
Decumbens	1.01	0.96	0.98 a
Brizantha	1.28	1.12	1.20 a
Humidicola	0.99	1.01	1.00 a
MG5	1.40	1.68	1.54 a
Ruzizienses	0.74	1.30	1.02 a
Mean	1.08 A	1.21 A	-
	2 nd Sampling		
Decumbens	2.07	2.33	2.20 ab
Brizantha	2.10	1.60	1.85 b
Humidicola	2.99	3.65	3.32 a
MG5	0.77	1.74	1.26 b
Ruzizienses	1.71	1.47	1.59 b
Mean	1.93 A	2.16 A	-
	3 rd Sampling		
Decumbens	2.10	2.98	2.54 a
Brizantha	2.88	2.36	2.62 a
Humidicola	1.79	2.90	2.34 ab
MG5	1.15	1.32	1.23 b
Ruzizienses	1.08	1.83	1.46 ab
Mean	1.80 A	2.28 A	-

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178 **4. CONCLUSION**

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180 1- Earthworm coprolites production is higher under pasture with B. MG5 during the dry
 181 season. In the rainy season, production increased under pasture with B. humidicola;

182 2- Brachiaria fertilized with liquid-enriched biofertilizer provided lower results in the
 183 production of biogenic aggregates (earthworm coprolites).

184 3- Under conditions of the present study, the hypothesis that the effect of the liquid -
 185 enriched biofertilizer increases the production of endogeic earthworm coprolites has not
 186 been proven.

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189 **COMPETING INTERESTS**

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191 Authors have declared that no competing interests exist.

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193 **CONSENT**

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195 It is not applicable

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197 **ETHICAL APPROVAL**

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199 It is not applicable

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201 **AUTHORS' CONTRIBUTIONS**

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203 *"This work was carried out in collaboration between all authors. Author A (Murielle Magda*
204 *Medeiros Dantas), B (José Flávio Cardoso Zuza) and C (Josinaldo da Silva Henrique)*
205 *managed the analyses of the study, performed the statistical analysis, and wrote the first*
206 *draft of the manuscript. Authors D (Joaquim Emanuel Fernandes Gondim) and E (Ana*
207 *Carolina Bezerra) managed the literature searches. Author E (Manoel Alexandre Diniz Neto)*
208 *designed the study and wrote the protocol. All authors read and approved the final*
209 *manuscript."*

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