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Production components of lettuce grown on drilocompost-based substrates of detritivorous earthworms

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Abstract

Alternative plant growing inputs have been increasingly sought out to reduce the economic-environmental impacts of intensive use of synthetic fertilizers in conventional agriculture. This study aimed to evaluate the production components of lettuce in response to the use of earthworm compost in substrate compositions. The experiment was carried out in a greenhouse using a completely randomized design in a 3 x 5 factorial scheme, with six replications, testing three earthworm species (*Eisenia andrei*, *Eudrilus eugeniae*, and *Perionyx excavatus*) and five drilocompost ratios (0%, 25%, 50%, 75%, and 100%), produced by these species and added to the composition of substrates by mixing them with soil. Production components evaluated comprised total and commercial numbers of leaves, commercial fresh weight, as well as shoot and total fresh and dry weights. Lettuce production components increased as the drilocompost ratio in substrates was raised, regardless of the earthworm species. Moreover, the addition of 25% this drilocomposted material in the dystrophic soil was enough to improve its fertility condition. In general, substrates prepared with worm castings from *Perionyx excavatus* and *Eudrilus eugeniae* were more efficient in increasing lettuce production components than that from *Eisenia andrei*.

Keywords: Organic farming, *Eisenia andrei*, *Eudrilus eugeniae*, *Lactuca sativa*, *Perionyx excavatus*

Introduction

Lettuce (*Lactuca sativa*) is the most produced, marketed, and consumed leafy vegetable in Brazil (Sala & Costa, 2012). Although this plant species is majorly grown in conventional and hydroponic systems, which require intensive chemical inputs, mainly nitrogen fertilizers, organic sources have become an alternative to reduce and/or replace industrial fertilizers (Echer et al., 2016). Moreover, lettuce production in organic systems may add economic value to the crop due to market demands for healthy foods and less environmental impacts from chemical fertilizers, especially nitrogen fertilizers (Lima et al., 2020).

Drilocompost, a term derived from the junction of *drilus* (earthworm in Latin) and compost (product from the composting process), also known as vermicompost, has been used in substrates and/or as a fertilizer source, which has brought positive effects to lettuce growth and production (Picazevicz et al., 2020; Steffen et al., 2010). The drilocomposting or vermicomposting process, which consists of organic compound ingestion and its passing through the guts of detritivorous earthworms, generates a material richer in nutrients and humic substances (Cotta et al., 2015). Plant positive responses promoted by drilocompost may be related to plant growth-stimulating substances released by these animals, as well as due to the increase of beneficial microbial diversity and phytopathogen control in drilocomposted materials (Brito-Vega & Espinosa-Victoria, 2009; Joshi et al., 2015).

Although the results of using organic materials processed by detritivorous earthworms are known, as well as their positive effects on fruit (Oliveira et al., 2013), vegetable (Armond et al., 2016), and forest (Silva et al., 2017) species, these are usually limited to *Eisenia andrei*, sometimes even erroneously named as *Eisenia fetida* since both have similar morphological traits, life cycle, and reproduction mechanisms (Domínguez & Pérez-Losada, 2010). Factors such as material

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source adaptability, tolerance to a certain temperature and humidity conditions, and high reproduction rate have increased the use of *Eisenia andrei* in drilocomposting to the detriment of other detritivorous species (Martín & Schiedeck, 2015). In this sense, our objective was to evaluate the potential use of drilocomposts produced by *Eisenia andrei*, *Eudrilus eugeniae*, and *Perionyx excavatus* in substrates and their effects on lettuce production components.

Material and Methods

The experiment was carried out in an arched metal-framed greenhouse, covered by 100-µm-thick low-density polyethylene, and on the sides, by 14x16-mm black polypropylene shade cloth. This structure is in the experimental field of the Federal University of Acre (9° 57' S latitude, 67° 52' W longitude, and 169-m altitude), in Rio Branco, Acre State, Brazil. The experimental period was from July to August 2015.

The lettuce cultivar Vera, of the *crespa* group, was used as it is resistant to early bolting under higher temperatures, such as in the study region. A completely randomized design was used in a 3 x 5 factorial scheme, with six replications, considering three earthworm species (*Eisenia andrei*, *Eudrilus eugeniae*, and *Perionyx excavatus*), which were used for processing the organic compost, and five ratios (0%, 25%, 50%, 75%, and 100%) of drilocompost for substrate composition.

Organic compost was produced by composting bovine manure, tropical kudzu (*Pueraria phaseoloides*), and bahiagrass (*Paspalum notatum*) at 1:1:2. These residues were deposited in alternating layers in a pile until it was 1.5 m high and 1.8 m wide. The mixture was kept at about 60% moisture for proper composting by aerobic decomposing microorganisms and thermophilic phase occurrence. To avoid water loss by direct evaporation or waterlogging by rains, the compost pile was covered with plastic tarpaulin. The pile was turned every 15 days to avoid compaction. The compost was suitable for use 90 days after mounting the pile when it was characteristically matured (Kiehl, 2004), therefore also bio-stabilized.

Drilocomposts were obtained from drilocomposting by detritivorous earthworms *Eisenia andrei*, *Eudrilus eugeniae*, and *Perionyx excavatus* in the organic compost previously produced. To do so, 5-kg transparent plastic boxes (bioprocessing units) were used, into which the organic compost and 15 animals were added and previously weighed to verify the biomass corresponding to the initial density, considering 10 containers for each earthworm species. The material was processed for 41 days while kept under suitable moisture conditions for earthworm survival. At the end of this time, the animals had fully processed the material, thus the drilocompost was obtained. The worms added, and later removed from the drilocomposted material, were subjected to evaluations for density, biomass, and recovery rates (Table 1).

Table 1. Density (unit) and biomass (g) of detritivorous earthworms added and recovered from organic compost bioprocessing units.

Species	Density		Total biomass		Mean biomass	
	Initial	Final	Initial	Final	Initial	Final
Eisenia andrei	150	209	34.55	51.76	0.23	0.25
Eudrilus eugeniae	150	188	50.93	72.72	0.34	0.39
Perionyx excavatus	150	178	56.53	79.73	0.38	0.45

The soil used for substrate compositions, classified as Red-Yellow Argisol soil, was collected from topsoil (0-10 cm) of a fallow area in the campus of the Federal University of Acre. Considering its initial chemical attributes (pH in H_2O = 4.80; Ca = 0.40 $cmol_c$ dm^{-3}; Mg = 0.20 $cmol_c$ dm^{-3}; K = 0.05 $cmol_c$ dm^{-3}; Al = 0.80 $cmol_c$ dm^{-3}; H+Al = 4.10 $cmol_c$ dm^{-3}; P= 0.02 $cmol_c$ dm^{-3}; CEC = 4.80 $cmol_c$ dm^{-3}; C = 8.97 g kg^{-1}; V = 14%; m = 55%), which was determined as proposed by Silva (2009), calcined lime (ENP 80%) was added to the soil at 0.5 t ha^{-1} to increase pH, reduce aluminum saturation and, at least, double base saturation. After 90 days of reaction, the soil was analyzed again (Table 2).

The drilocomposts from *Eisenia andrei*, *Eudrilus eugeniae*, and *Perionyx excavatus* were mixed to the soil at 25%, 50%, and 75% ratios to compose substrates for lettuce cultivation, also considering substrates with 100% soil and 100% drilocompost of each earthworm species. Volumetric references were established for each drilocompost ratio added to the soil (25%, 50%, and 75%), considering each experimental unit characteristics, which consisted of a white polyvinyl chloride (PVC) pot, measuring 15 cm in diameter, 14.5 cm in height, and 2 L in volume. The chemical characterization of the substrates is presented in Table 2.

Lettuce seedlings were produced on a phenolic resin foam. Ten days after sowing, when they had 2 permanent leaves, the seedlings were transferred to the experimental units, planting two seedlings per pot. The seedlings were thinned out 10 days after transplanting, maintaining one plant per pot, which was considered the most vigorous. The plants were regularly irrigated manually to maintain substrates at about 70% of the field capacity. During the experimental period, temperature and relative humidity were monitored by a datalogger installed inside the greenhouse, and the averages obtained were 33 °C and 67%, respectively.

Table 2. Chemical characterization[1] of the soil and substrates at different drilocompost ratios (25%, 50%, 75%, and 100%) from *Eisenia andrei (Ea)*, *Eudrilus eugeniae (Ee)*, and *Perionyx excavatus (Pe)*.

Substrate		pH	Ca	Mg	K	Al	H+Al	CEC	P	C	V	M
			.... $cmol_c\ dm^{-3}$						$mg\ dm^{-3}$	$g\ kg^{-1}$	.... %	
Soil	100%	5.30	1.25	0.50	0.05	0.30	4.10	6.00	0.04	12.17	32	14.2
Ea	25%	5.28	3.36	3.63	4.37	0.09	3.20	14.98	1.33	10.94	77	0.8
	50%	6.51	5.53	6.24	10.48	0.20	2.66	25.34	4.46	17.01	90	0.9
	75%	6.87	6.48	8.47	16.10	0.24	2.87	35.42	9.35	35.68	92	0.7
	100%	7.22	8.60	12.45	15.98	0.35	3.33	41.76	18.25	61.32	92	0.9
Ee	25%	5.50	3.58	3.45	3.30	0.13	3.36	13.91	1.27	8.80	77	1.2
	50%	6.31	5.95	6.02	7.46	0.27	2.98	23.14	3.74	17.06	87	1.3
	75%	6.77	6.98	7.92	10.48	0.33	3.19	29.75	8.89	34.38	89	1.2
	100%	6.90	9.83	12.08	10.92	0.50	3.50	37.46	17.83	58.99	90	1.5
Pe	25%	5.41	7.75	11.05	3.26	0.11	3.54	15.75	2.16	12.71	78	0.9
	50%	6.21	6.13	5.68	6.68	0.15	2.70	21.68	5.83	25.68	88	0.8
	75%	6.68	8.23	8.52	12.77	0.17	2.78	33.75	11.65	35.75	92	0.6
	100%	6.88	10.9	11.92	13.95	0.25	3.72	42.33	24.17	71.52	91	0.6

[1] Chemical analysis method to assess substrate fertility according to Silva (2009).

The experiment was evaluated 38 days after transplanting seedlings when at least 50% of the plants showed maximum vegetative development. After plants were removed from the pots, they were evaluated for total and commercial leaf numbers, commercial fresh weight, and shoot and total fresh and dry weights. The number of leaves was obtained by counting and expressed in units. Fresh and dry weights were determined using a digital scale with 0.01 g precision. Shoot and total fresh weights considered all leaves, regardless of their condition. However, for commercial weight, non-standard leaves (senescent, stained, with physical damage) were dis-regarded. Dry weights were obtained after keeping fresh parts in an oven at 65 °C until a constant weight was reached and measured on a scale.

The results were analyzed to determine the presence discrepant measurements (Grubbs, 1969), error normality (Shapiro & Wilk, 1965), and variance homogeneity (Cochran, 1941). F-test analysis of variance was used to verify the significance of isolated and/or combined effects of the factors analyzed. In the event of significant interactions for one or both factors, a regression analysis was performed for the quantitative factors (drilocompost ratio) and/or Tukey's test (1949) for qualitative ones (earthworm species). For variables whose interaction between factors was significant ($p < 0.05$), the degrees of freedom of the treatments were unfolded, considering the effects of levels of one within the other. Moreover, by orthogonal contrast (Nogueira, 2004), the effects on the evaluated variables in substrates with 100% soil were verified and compared to those with the minimum vermicompost ratio (25%), regardless of the earthworm species. Analysis of variance was performed using the Sisvar statistical software (Ferreira, 2011).

Results and Discussion

An interaction effect ($p < 0.05$) between the factors was found for lettuce production components. Total and commercial leaf numbers (Table 3), as well as shoot, commercial, and total plant fresh weights (Table 4), increased linearly as the drilocompost ratio increased and soil amount reduced in the substrates, regardless of the earthworm species. However, when comparing the species for each drilocompost ratio, the performance of the evaluated variables was generally superior in those produced by *Perionyx excavatus* and *Eudrilus eugeniae* (Tables 3 and 4). Therefore, if compared to *Eisenia andrei*-derived drilocompost, the best responses were observed in plants grown on substrates with *Perionyx excavates*- and *Eudrilus eugeniae*-derived drilocomposts.

The largest increments in shoot and total dry weights of lettuce plants were derived from the isolated effect ($p < 0.05$) of earthworm species, regardless of the drilocompost ratio in the substrate (Table 5). For both parameters, the species *Eudrilus eugeniae* and *Perionyx excavatus* stood out with respective shoot dry weights 24.38% and 19.01% higher than those observed for *Eisenia andrei*. As for total dry weight, increments were 20.98% and 14.69% greater than those with *Eisenia andrei*-derived drilocompost. These results suggest that *Eudrilus eugeniae*- and *Perionyx excavatus*-derived drilocomposts provided the best conditions for lettuce growing. According to Kusdra and Fiuza (2015), earthworms and therefrom derived products have different effects on soil and plants growing on it. However, these authors stated that detritivorous species-derived drilocomposts, such as in this experiment, usually have positive effects on plants.

Table 3. Total (TLN) and commercial (CLN) leaf numbers of lettuce as a function of the interaction between drilocompost ratio in substrates and the earthworm species *Eisenia andrei* (*Ea*), *Eudrilus eugeniae* (*Ee*), and *Perionyx excavatus* (*Pe*).

Variable	Earthworm	Drilocompost ratio					Equation	R^2	CV
		0%	25%	50%	75%	100%			
TLN	*Ea*	3.83a	10.00b	9.33b	12.33c	17.00b	y = 0.114x + 4.766	0.8965**	11.85%
	Ee	3.33a	11.17b	11.50a	17.67a	20.33a	y = 0.162x + 4.700	0.9405**	
	Pe	3.67a	13.17a	11.33a	14.83b	20.50a	y = 0.141x + 5.633	0.8374**	
CLN	*Ea*	0.00a	8.33b	7.67b	10.50c	15.00b	y = 0.128x + 1.866	0.8695*	12.10%
	Ee	0.00a	9.83b	9.67a	12.67b	18.00a	y = 0.155x + 2.266	0.8808**	
	Pe	0.00a	11.67a	9.83a	15.83a	18.50a	y = 0.164x + 2.934	0.8376**	

Means followed by the same letter in the columns do not differ (p > 0.05) according to the Tukey's test.

Table 4. Shoot (SFW), commercial (CFW), and total (TFW) fresh weights of lettuce as a function of the interaction between drilocompost ratio in substrates and the earthworm species *Eisenia andrei* (*Ea*), *Eudrilus eugeniae* (*Ee*), and *Perionyx excavatus* (*Pe*) .

Variable	Earthworm	Drilocompost ratio					Equation	R^2	CV
		0%	25%	50%	75%	100%			
SFW (g)	*Ea*	0.17a	28.55a	23.47a	52.63b	105.06b	y = 0.936x - 4.862	0.8598**	30.83%
	Ee	0.19a	39.76a	29.56a	62.47b	142.22a	y = 1.227x - 6.526	0.8151*	
	Pe	0.06a	41.17a	42.74a	98.40a	162.47a	y = 1.527x - 7.364	0.9226**	
CFW (g)	*Ea*	0.00a	27.65a	22.84a	50.19b	99.44b	y = 0.885x - 4.260	0.8626**	30.97%
	Ee	0.00a	37.92a	28.79a	60.45b	132.93a	y = 1.153x - 5.660	0.8266**	
	Pe	0.00a	40.25a	41.17a	91.92a	153.43a	y = 1.434x - 6.352	0.9215**	
TFW (g)	*Ea*	0.19a	33.70a	27.31a	58.58b	116.17b	y = 1.028x - 4.244	0.8599**	29.79%
	Ee	0.20a	48.02a	35.39a	68.32b	150.75a	y = 1.285x - 3.750	0.8180**	
	Pe	0.08a	51.08a	51.89a	107.40a	173.24a	y = 1.609x - 3.718	0.9313**	

Means followed by the same letter in the columns do not differ (p > 0.05) according to the Tukey's test.

Table 5. Shoot (SDW) and total (TDW) dry weights of lettuce as a function of the drilocomposts produced from the earthworm species *Eisenia andrei* (*Ea*), *Eudrilus eugeniae* (*Ee*), and *Perionyx excavatus* (*Pe*), regardless of the drilocompost ratio used in substrate.

Variable	Earthworm species			CV
	Ea	*Ee*	*Pe*	
SDW (g)	2.42b	3.01a	2.88ab	24.38%
TDW (g)	2.86b	3.46a	3.28ab	20.98%

Means followed by the same letter in the rows do not differ (p > 0.05) according to the Tukey's test.

Lettuce shoot and total dry weights were also positively influenced to the increases in drilocompost ratio in substrate, regardless of the earthworm species. Despite a quadratic behavior, it resembled linear throughout the drilocompost ratios studied (0 to 100%). Therefore, increases in these variables were proportional to progressive improvement in substrate chemical conditions (Table 6). Lettuce growth, therefore, was favored by adding drilocompost to the soil regardless of the ratio. Such increments in shoot and/or total dry weight in lettuce grown on substrates with drilocompost mixed with the soil were also observed by Picazevicz et al. (2020) and Teodoro et al. (2016).

Table 6. Shoot (SDW) and total (TDW) dry weights of lettuce as a function of the drilocompost ratio in substrate composition regardless of the earthworm species.

Variable	Drilocompost ratio					Equation	R^2	CV
	0%	25%	50%	75%	100%			
SDW (g)	0.01	2.12	1.80	3.59	6.33	$y = 0.0004x^2 + 0.018x + 0.426$	0.9281**	27.86%
TDW (g)	0.01	2.64	2.25	4.14	6.96	$y = 0.0003x^2 + 0.031x + 0.500$	0.9165**	27.03%

The organic composts processed by *Eisenia andrei*, *Eudrilus eugeniae*, and *Perionyx excavatus* contributed to increase by 39.33%, 25.33%, and 18.67% their densities and by 49.81%, 42.78%, and 41.04% their final biomasses, respectively (Table 1). According to Dionísio (2020), organic material is considered ideal for drilocomposting when it ensures earthworm survival and reproduction. Thus, the characteristics of the compost used as a reference for obtaining the drilocomposts were adequate not only to ensure the survival of the three earthworm species but also to allow an increase in their densities and biomass. Furthermore, the chemical analysis of the drilocomposted material (Table 2) showed fertility characteristics with potential to produce positive effects on the production components of lettuce.

Incorporation of drilocomposts into soil increased pH and reduced aluminum saturation, besides increasing macronutrient content, cation exchange capacity, and base saturation, which, according to Filgueira (2008), are suitable for plant cultivation. As the chemical conditions of the soil used in substrate formulations could not supply the nutritional requirements of lettuce, only after incorporation of the drilocomposted material, their fertility was improved (Table 2). Therefore, lettuce production components were positively influenced by the progressive improvement in substrate nutritional quality as the ratios of soil in its composition were reduced and of drilocompost increased. Improvements in soil chemical properties (N, P, K, pH, and organic matter) were also observed by Hoehne et al. (2020), using different ratios (20%, 40%, 60%, 80%, and 100%) of organic material processed by *Eisenia andrei*. Likewise, Steffen et al. (2010), observed a greater initial growth of lettuce in substrates with a gradual replacement of soil by drilocompost from *Eisenia andrei*.

Considering the drilocompost ratios evaluated (25%, 50%, and 100%), the highest one allowed plants to express their full growth potential. However, when compared to soil substrate without drilocompost (0%), the addition of these material, even at the smallest ratio (25%) and regardless of the earthworm species (*Eisenia andrei*, *Eudrilus eugeniae*, or *Perionyx excavatus*), resulted in significant increases in total and commercial leaf numbers, as well as shoot, commercial, and total fresh weights of lettuce plants (Table 5). This is because substrate chemical properties had already been improved by adding the lowest vermicompost ratio (25%) to the soil (Table 2). Durak et al. (2017) and Manyuchi et al. (2013) evaluated drilocompost feasibility in conditioning soil chemical properties and observed increases in nutrient and organic matter contents with only small amounts of this material, and also reported positive effects on plants similar to those by chemical fertilizers. Thus, using vermicomposts as alternative inputs to improve soil chemical quality can reduce and/or replace the application of synthetic sources to supply lettuce nutritional demands.

Table 7. Comparison by orthogonal contrast between the substrates with 100% soil and with 25% drilocompost, regardless of the earthworm species, regarding total (TNL) and commercial (CNL) leaf numbers, as well as shoot (SFW), commercial (CFW), and total (TFW) fresh weights of plants of the cultivar Vera lettuce.

Substrate	TNL	CNL	SFW	CFW	TFW
	.... unit		.... g		
Soil (100%)	3.61b	0.00b	0.14b	0.00b	0.16b
Soil (75%) + vermicompost (25%)	11.45a	9.94a	36.49a	35.27a	44.27a
CV (%)	11.85	12.10	30.83	30.97	29.79

Means followed by the same letter in the column do not differ ($p > 0.05$) according to the F-test.

The effects observed on lettuce in this study indicate that drilocomposts produced by different earthworm species can interfere differently with the production components of plants. On the one hand, the evaluated variables improved progressively as the soil was replaced with drilocompost (25% to 100%), regardless of the earthworm species. On the other hand, when evaluating each ratio individually, the best responses were, in general, observed in drilocomposts from *Eudrilus eugeniae* and *Perionyx excavatus*. Therefore, these two species may produce drilocomposts richer than those from *Eisenia andrei* in terms of compounds and microorganisms capable of promoting plant growth. According to Brito-Vega & Espinosa-Victoria (2009) and Domínguez et al. (2019), as organic residues pass through the digestive tract of detritivorous earthworms, plant growth-promoting substances and microorganisms can be increased, speeding up nutrient cycling and release, and thus enhancing benefits to plant growth and production.

Although *Eudrilus eugeniae*- and *Perionyx excavatus*-derived drilocomposts performed better, the one from *Eisenia andrei* produced similar effects as observed to these former species on total and shoot fresh weights when applied at a ratio of 50% (Table 4). It is important to highlight, however, that among the three materials, *Eisenia andrei*-derived drilocompost has been the most used in substrates not only for growing lettuce but also for other plant species (Armond et al., 2016; Hoehne et al., 2020; Silva et al., 2017; Steffen et al., 2010). According to Martín and Schiedeck (2015), some factors justify the frequent use of *Eisenia andrei* for drilocomposting, such as adaptability to different material sources, tolerance to different temperature and humidity ranges, and its high reproduction rate.

Both *Eudrilus eugeniae* and *Perionyx excavatus* were efficient in processing organic wastes and, as substrate components, their drilocomposts had positive effects on lettuce production, increasing leaf number and fresh weights (Table 3 and 4). Thus, both species can, as well as *Eisenia andrei*, be used to prepare drilocomposts and then use in growth substrates for lettuce cultivation to promote productivity gains.

Other studies have already shown promising results for earthworm drilocomposts, especially those derived from *Eudrilus eugeniae* and *Perionyx excavatus*. Picazevicz et al. (2020) obtained significant lettuce yield gains with the use of *Eudrilus eugeniae* drilocompost, with responses like those of inorganic NPK application into the soil. Singh et al. (2014)

stated that *Perionyx excavatus* is an efficient species in organic waste processing and contri-butes to an increase in the potassium content, carbon mineralization, and microbial activity in drilocomposted material.

In general, all drilocomposts showed fertility characteristics that increased the production components of lettuce. Furthermore, regardless of the earthworm species and drilocompost ratio, the substrates were also more suitable than the soil in producing positive effects for the plants. Therefore, there is potential for incorporating the three drilocomposts in lettuce growth substrates, as they tend to improve the production performance of the cultivar Vera, even if in small quantities.

Conclusions

Adding 25% *Eisenia andrei*-, *Eudrilus eugeniae*-, and *Perionyx excavatus*-derived drilocomposts to dystro-phic soil is enough to obtain suitable substrates for gro-wing lettuce.

Substrates prepared with *Perionyx excavatus*- and *Eudrilus eugeniae*-derived drilocomposts are more efficient than those with *Eisenia andrei*-derived material in promoting increases in the production components of lettuce.

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