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ORIGINAL RESEARCH

Effect of substrates consisting of organic waste processed by diplopods on production of lettuce seedlings

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Abstract

Purpose To characterize substrates made of millicomposts produced from a mixture of cow paw leaves (*Bauhinia* sp.), grass clippings (*Paspalum notatum*), banana leaves (*Musa* sp.) and cardboard submitted to processing through the activity of millipedes *Trigoniulus corallinus* for 90, 125 or 180 days. The efficiency of producing lettuce seedlings using these substrates was compared to a control substrate of earthworm humus, fine coal and castor cakes.
Method Chemical and physical characteristics of the substrates, seedling growth and clod stability were evaluated 28 days after sowing. Substrate pH and electrical conductivity during seedling development were also recorded.
Results The millicompost with the shortest processing time (90 days) contained lower levels of macronutrients (N, Ca, Mg, P and K) compared to the millicomposts with 125 and 180 days of processing and the control substrate. Subsequently, the lettuce seedlings produced on 90-day substrate showed less vegetative development. The millicomposts with 125 or 180 days of processing did not differ from the control substrate in respect to lettuce seedling development. Clod stability was greater in the control and the 180-day millicompost substrates.
Conclusion The results show that it is possible to obtain efficient substrates for lettuce production using a mixture of various plant residues with cardboard processed through the activity of millipedes for a minimum of 125 days.

Keywords Diplopoda, Millicomposting, Organic wastes, *Lactuca sativa* L., Vegetables

Introduction

The use of locally available organic composition materials, such as by-products from agricultural or agro-industrial production, is considered an important source of raw materials for the production of substrates, by reducing the input costs (Campanharo et al. 2006; Silva et al. 2009; Souza et al. 2013), in addition to providing a more suitable destination for the waste (Costa et al. 2015). Composting technology fits into this scenario, transforming plant and animal waste into stable organic matter, through a process promoted by a variety of saprophagous fauna organisms and microorganisms (Ambarish and Sridhar 2013). Among these organisms, earthworms are efficient in the transformation of animal waste (Oliveira et al. 2013), while millipedes can be used in the processing of diverse plant waste, even the most recalcitrant in nature (Ramanathan and Alagesan 2012; Antunes et al. 2016; Antunes et al. 2019).
Earthworms are the most well-known composting promoting organisms, and their role as a waste stabilizer is considered ideal (Dores-Silva et al. 2013). The activity of these organisms ingesting decomposing materials, subjecting them to churning and crushing in their digestive tract, where there are associated decomposing microorganisms, and excreting humidified organic matter (Garg and Yaday 2011), generates a compost with a greater capacity for exchange of cations, moisture retention, intensifying its beneficial effects on plants (Aquino et al. 1992; Dores-Silva et al. 2013; Cotta et al. 2015).

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Different to the classical composting, which presents different temperature phases (mesophilic and thermophilic), millicomposting consists of a composting process without thermal variation, based on the activity of diplopods, which are popularly known as millipedes, playing important pedogenetic processes in the cycling of soil nutrients, fragmentation and transformation of organic matter and in the composting of plant residues (Ramanathan and Alagesan 2012). Diplopods transform plant material into fecal pellets, which influences important physical and chemical properties, such as decreased C/N ratio and increased humic substances (Thakur et al. 2011; Karthigeyan and Alagesan 2011; Ramanathan and Alagesan 2012). Seen as a compost, the residue generated by the activity of the millipedes received the name of millicompost and has important properties in terms of nutrition and microflora (Kania and Klapec 2012). A species with great potential for this purpose is *Trigoniulus corallinus* (Gervais), originally from Southeast Asia, which has a pantropical distribution, being easily found in agricultural environments, mainly due to its distinctive intense red color (Shelley et al. 2006; Bianchi and Correia 2007).

Earthworm humus is already recognized for its great potential to be used in the formulation of substrates used in the production of seedlings in organic production systems, mainly due to the easy access to raw material from livestock activities (Steffen et al. 2010; Oliveira et al. 2013). The substrates formulated with residues from the activity of diplopods are little known; however, they also have great potential for the production of lettuce seedlings (*Lactuca sativa* L.) (Antunes et al. 2016).

Research results, as demonstrated by Antunes et al. (2018), demonstrate that it is possible to obtain millicompost from 90 days, but longer processing times may be necessary to obtain better quality substrates, because as time passes, the greater the transformation of the waste or by-product of composition organic in stable organic matter is. However, the minimum processing time to obtain efficient substrates has not yet been established.

The objective of this work was to characterize millicompost produced from the mixture of plant residues and cardboard shavings, submitted to different processing times through the activity of *Trigoniulus corallinus* (Gervais) millipedes, and to evaluate their efficiency as substrates for the production of lettuce seedlings.

Material and methods

The production of the millicompost and a seedling growth experiment were carried out in the Integrated System of Agroecological Production (SIPA - "Fazendinha Agroecológica Km 47"), located in Seropédica, RJ, Brazil. The climate in the region is an Aw type, according to the Köppen classification, where rains are concentrated between November and March, and an average annual temperature of 23.9 °C and precipitation of 1,213 mm (Oliveira Júnior et al. 2014).

Production of millicompost

The production of millicompost started in August 2015 and was completed in February 2016, being carried out with diplopods of the species *Trigoniulus corallinus* (Gervais). Three concrete rings were used, with a height of 0.5 m and a diameter of 1 m, with a capacity to receive approximately 400 L of waste. In the first stage, the residues were quantified and deposited inside the three rings, at a height of approximately 40 cm. Residues from *Bauhinia sp*. (cow paw leaves), *Paspalum notatum* (grass clippings), *Musa sp.* (banana leaves) and cardboard shavings in the proportion of 200 L, 150 L, 100 L and 50 L, respectively. The proportion of each waste used was based on the consumption results obtained by Antunes et al. (2019), which found during the ten-day period higher consumption of banana leaves, followed by cow paw leaves, grass clippings and cardboard. The higher proportion of cow's paw leaves was motivated by the fact that it had higher nitrogen content, resulting in a more nutrient-rich compound.

The waste was wetted and homogeneously mixed. In the second stage, each ring received an approximate amount of 2.2 L of millipedes, which is equivalent to a population of approximately 3,960 adult individuals, collected manually in vermicomposting beds, in compost pans and on the lawn containing fresh cuttings. The maintenance of humidity was performed with the addition of water via a watering can, weekly or when necessary. Then, the rings were covered with a shade to prevent millipedes from escaping when climbing the wall of the ring or preventing something unwanted from entering the ring, impairing the process of millicomposting. The contents of macronutrients present in the residues used are shown in Table 1.

Table 1 Carbon/nitrogen ratio (C/N) and macronutrient content in materials used for millicomposting

Residue	C/N Ratio	N	P	K	Ca	Mg
		------------------------- g kg^{-1} --------------------------				
Cow paw	39.62	12.80	0.90	2.75	34.52	3.59
Grass	25.22	20.20	3.49	19.84	4.17	3.12
Banana	56.02	9.40	0.65	13.21	11.62	2.89
Cardboard	291.04	1.90	0.20	0.52	5.89	0.50

The millicomposts were obtained at 90, 125 and 180 days after starting the above process. After being removed from the rings, the millicomposts were sieved in 2.0 mm mesh and stored in plastic bags, which remained frozen in order to paralyze biological activity, being thawed only at the specific time to be used in the production of vegetable seedlings, as a substrate.

Characterization of substrates

A detailed characterization of the chemical and physical properties of the substrates (millicomposts of 90, 125 and 180 days) and the control called substrate SIPA (83% of vermicompost produced in the Integrated System of Agroecological Production + 15% fine plant charcoal and 2% castor bean cake), as proposed by Oliveira et al. (2011), was carried out, with three replications for all evaluated parameters. Total levels of N, P, K, Ca and Mg were obtained according to the methodology described by Teixeira et al. (2017). The determination of the carbon content was made in the elemental analyzer (CHN), also known as Dumas' method (Nelson and Sommers 1996).

The characterization of N forms present in the substrates was also carried out: N-ammonium, N-nitric and N-organic. For this analysis, twenty grams of substrate were subjected to extraction with 60 mL of K_2SO_4 2 moles L-1 for one hour on a rotary shaker at 220 rpm. The supernatant was filtered and the concentrations of NO_3^- and NH_4^+ were determined in the resulting solution by UV spectrometry. For the determination of NO_3^-, the procedures described by Miyazawa et al. (1985) were used, but using only 220 and 275 nm wavelengths. The absorbance at 275 nm was multiplied by two and then subtracted from the absorbance at 220 nm to determine the NO_3^- absorbance, as described by Olsen (2008). For the determination of NH_{4+}, the salicylate-hypochlorite procedure was used (Kempers and Zweers 1986). Organic N was obtained by subtracting N-ammonium and N-nitrate from total N.

As for the physical characteristics, the values of volumetric density, macroporosity, microporosity and total porosity were determined, as well as the water retention capacity through the tension table methodology described by Teixeira et al. (2017).

Experiment with lettuce seedlings

The production of lettuce seedlings was carried out in expanded polystyrene trays with 200 cells. Two pelleted seeds of the curly lettuce cultivar Vera were placed in each cell. After nine days, the seedlings were thinned to one plant per cell. Each experimental unit consisted of a tray.

pH and electrical conductivity during the development of lettuce seedlings

Sampling for measurements of pH and electrical conductivity (EC) was performed at 0, 7, 14, 21 and 28 days after sowing. Substrate from four randomly selected cells was collected per experimental unit each time, totaling 50 mL of substrate. The pH and EC analyses were performed in a distilled water/substrate solution (5:1 v/v), according to the method described by Brasil (2008).

Statistical analysis was performed using the subdivided plot scheme, with substrate as the main plot and seedling development times as the sub-plot, in a completely randomized design with four replications. The variations in pH and electrical conductivity throughout the development of the seedlings were presented by means of graphs containing the average values and the standard error.

Evaluation of lettuce seedlings

At 28 days after sowing, ten lettuce seedlings were removed at random per experimental unit and were evaluated: the fresh mass of the aboveground plant (FMAP), dry mass of the aboveground plant (DMAP), fresh mass of the roots (FMR), dry mass of the roots (DMR), plant height (PH), number of leaves (NL), seedling vigor (SV) and clod stability (CS). After weighing the fresh mass of the aerial part and the roots, they were individually separated in paper bags and kept in a forced air circulating oven at 65 °C for 72 hours, in order to determine their dry mass.

Seedling vigor (SV) was determined using the methodology adapted from Franzin et al. (2005), classifying as Note 1: excellent vigor, number of leaves ≥ 4, a height greater than 5 cm and visual absence of nutritional deficiency; Note 2: good vigor, number of leaves ≥ 4, height ≥ 5 cm and yellowish beginning not prominent in the basal leaves; Note 3: regular vigor, number of leaves ≥ 4, height ≥ 5 cm; nutritional deficiency expressed by a prominent yellowing that extends beyond the basal leaves or other intrinsic symptoms; Note 4: poor vigor, well-defined nutritional deficiency, expressed by height problems (≤ 5 cm), reduced number of leaves (≤ 4 leaves) and intense yellowing or other intrinsic symptoms.

Clod stability (CS) was determined by a methodology adapted from Gruszynski (2002), classifying as Note 1: Low stability, 50% or more of the substrate is retained in the container when the seedling is removed and the clod does not remain cohesive; Note 2: Between 30 and 50% of the substrate is retained in the container when the seedling is removed but the clod does not remain cohesive; Note 3: Regular, between 15 to 30% of the substrate is retained in the container when the seedling is removed, but does not remain cohesive; Note 4: Good stability, the clod is completely detached from the container with up to 90% cohesion and maximum loss of up to 10% of the substrate.

The design used was randomized blocks with four replications, where four treatments (substrates) were evaluated. For data analysis, evaluations were made of the homogeneity of the error variances by the Bartlett test and normality by the Shapiro-Wilk test. The data were subjected to analysis of variance by the F test and subsequently submitted to the Scott-Knott test of means (≤0.05), using the Rbio statistical program (Bhering 2017).

Results and discussion

Physico-chemical and chemical characteristics of substrates

The total carbon content of the substrates ranged from 276 to 379 g kg^{-1} (Table 2). Also in Table 2, it is possible to notice that the substrates S1, S2 and S3 (millicomposts) showed decreasing C/N ratio and C total values and increases in macronutrient contents, respectively, according to the time of obtaining the substrate. Total N was similar among the substrates (Fig. 1). However, N available as ammonium and nitrate was significantly higher for the control substrate (S4). The substrate S1 showed 99% of its N in organic form, which is not available to plants. The other substrates had organic N content ranging from 92.88 (S2) to 81.17% (S4).

The content of other nutrients (P, K, Ca and Mg) also increased according to the millicomposting time for the substrates S1, S2 and S3. Due to the type of waste used in millicomposting, more time is needed for stabilization and thus improving its chemical properties. The content of nutrients in the S4 substrate was higher, while the content of calcium was lower than in all millicomposts. Schmitz et al. (2002), assuming 50 to 60% of organic matter is carbon, established that more

Table 2 Analysis pH, electrical conductivity (EC), C/N ratio, total carbon content and total macronutrients of the substrates evaluated in the production of curly lettuce seedlings cv. Vera

Substrates	C/N Ratio	C g kg^{-1}	N	P	K	Ca	Mg
				------------------- mg L^{-1} --------------------			
S1 - Millicompost 90 days	18.98 a	379 a	9133 c	617 d	2970 d	12872 c	2215 c
S2 - Millicompost 125 days	16.51 b	357 b	10994 b	801 c	3724 b	16125 b	2727 b
S3- Millicompost 180 days	15.05 c	352 b	10694 b	996 b	3341 c	16868 a	2766 b
S4 -SIPA	16.65 b	276 c	11996 a	3922 a	5931 a	10549 d	5059 a
CV (%)	2.15	1.16	2.12	4.29	3.05	9.12	4.94

Means followed by the same letter in the column do not differ by the Scott-Knott test (≤0.05)
SIPA substrate: 83% of vermicompost + 15% fine plant charcoal and 2% castor bean cake

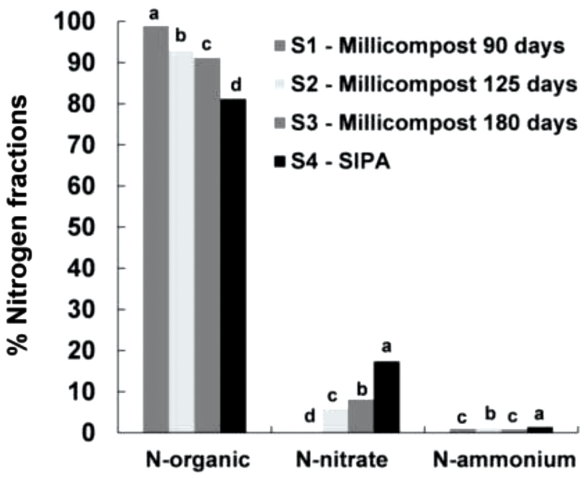

Fig. 1 Proportion (%) of different forms of nitrogen present in the substrates used to produce curly lettuce cv. Vera

Means followed by the same letter in the bar do not differ by the Scott-Knott test (≤0.05)

SIPA substrate: 83% of vermicompost + 15% fine plant charcoal and 2% castor bean cake

than 250 g kg^{-1} organic carbon is ideal for container substrates. All the substrates used in this work fit the range of 276 to 379 g kg^{-1} (Table 2).

The results of C/N ratio, organic carbon and nutrients obtained in this work corroborate the results found by Ramanathan and Alagesan (2012), who found decreases in the C/N ratio and total C with increases in the levels of N, P, K and Ca for the millicompost produced in 60 days. Antunes et al. (2016) also observed the nutritional enrichment of the millicompost with Ca and Mg, being effective in the production of Regina 2000 lettuce seedlings. The C/N ratio is an important parameter for characterizing the substrate, as it indicates the form N at the end of the composting process (Da Ros et al. 2015). The Normative Instruction number 25 of the Ministry of Agriculture, Livestock and Supply (MAPA 2009) highlights that the C/N ratio cannot exceed 20 and the total nitrogen content must be at least 5.0 g kg^{-1} for organic composts. In this way, all the substrates used in this work meet the requirements of the normative instruction.

The lower C/N ratio of substrates S2, S3 and S4 (Table 2) promoted a greater availability of mineral N (Fig. 1), resulting from the mineralization of organic N which, according to Pereira (2013), has an inverse relationship between the C/N ratio and nitrogen availability in the substrate. Similar results were observed by Da Ros et al. (2015), who evaluated the germination, growth and quality of *E. dunnii* and *C. trichotoma* seedlings in different substrates from the composting of organic residues, finding the greatest growth of seedlings in the substrates with the lowest C/N ratio (18.6 to 29.3).

Nitrogen (N) is an essential element for plants and low levels present in the substrates directly affects the formation of roots, the process of photosynthesis, the production and translocation of photoassimilates and the growth rate between leaves and roots, with leaf growth being primarily affected (Taíz and Zieger 2004). The substrates had similar total levels of N; however, the proportions of available N varied, mainly in relation to the substrate S4, where the proportion N-ammonium + N-nitrate was higher than in the other substrates (Fig. 1). The substrate S1 showed 99% of N in organic form that is not available to plants, which may explain, together with the high pH, the reduced initial seedling development supported by this substrate.

Currently, there are no ideal nutrient ranges for substrates in the literature. However, Gonçalves and Poggiani (1996) established value scales for the interpretation of substrate chemical characteristics, such as adequate levels of macronutrients. The concentration of phosphorus considered adequate varies from 400 to 800 mg L^{-1}, but it was above that established only for substrates S3 and S4 (Table 2).

Potassium levels between 1173 to 3910 mg L^{-1} are considered adequate and only substrate S4 had above the established levels (Table 2). The calcium levels were above the levels considered adequate (2004 at 4008 mg L^{-1}) for all substrates, mainly for substrates S2 and S3 (Table 2). This significantly greater difference in these substrates is due to the low survival rate of the millipedes until the end of the millicomposting process, which promoted the incorporation of the nutrients present in their bodies into the compost, mainly calcium. All substrates also showed Mg above the adequate levels

(607 to 1215 mg L^{-1}), with substrate S4 superior to the other substrates (Table 2). This superiority in the values of N, P, K and Mg in the substrate S4 is attributed to bovine manure as a raw material for vermicomposting. However, the substrates S1, S2 and S3 (millicomposts), meet or are above the appropriate ranges established by the aforementioned authors, which proves to be effective in providing nutrients to plants.

Physical characteristics of substrates

The Table 3 shows the physical characteristics that were evaluated on the substrates used in this experiment. The volumetric density was different for the four substrates, with millicomposts S3 being the least dense (220 kg m^{-3}).

The millicomposts had the highest total porosity, with substrate S3 having the highest percentage (89.59%) while S4 had the lowest percentage (76.47%). The highest percentages of microporosity were recorded for substrate S3 (64.97%), followed by substrates S1 and S2 (50%) and S4 with 48.16%. The macroporosity differed statistically only for the substrate S3 (24.63%), which was lower in relation to the other substrates, whose macroporosity varied from 28.31 to 30.17%. The water retention capacity was higher for the substrate S3 while the control substrate S4, showed the lowest retention capacity at 24.08 mL 50 cm^{-3}.

The density of a substrate is important for porosity, aeration and water availability (Fermino 2003). A density between 100 and 300 kg m^{-3} is considered as a reference for substrates used in trays (Fermino 2002). Thus, the substrates S1, S2 and S3 (millicomposts) present densities within the range proposed for use in trays, with only the substrate S4 having a volumetric density above the proposed standard, probably due to the addition of fine coal in the formulation of this substrate. Furthermore, the density is an important property for management. In addition to management, density influences cost of transport, handling and required infrastructure (Fernandes et al. 2006).

Table 3 Physical analysis of the evaluated substrates: volumetric density, percentages of total porosity, microporosity, macroporosity and water retention capacity at a tension of 10 cm of water column (CRA10 cm)

Substrates	Volumetric density (kg m^{-3})	Porosity (%)			$CRA_{10\ cm}$ (mL 50 cm^{-3})
		Total	Micro	Macro	
S1 - Millicompost 90 days	240 c	80.00 b	50.09 b	30.17 a	25.04 b
S2 - Millicompost 125 days	320 b	80.26 b	50.74 b	29.26 a	25.37 b
S3- Millicompost 180 days	220 d	89.59 a	64.97 a	24.63 b	32.48 a
S4 -SIPA	370 a	76.47 c	48.16 c	28.31 a	24.08 c
CV (%)	0.74	1.28	1.85	4.47	1.85

Means followed by the same letter in each column do not differ by the Scott-Knott test (≤0.05). SIPA substrate: 83% of vermicompost + 15% fine plant charcoal and 2% castor bean cake.

According to the values indicated as adequate for total porosity by Gonçalves and Poggiani (1996), the substrates S1, S2 and S4 are considered adequate (Table 3), standing in the range of 75 to 85%. Although the substrate S3 had a higher total porosity than the other substrates, it meets the recommendation of Carrijo et al. (2002), being above 85% (Table 3). According to Kämpf (2005), the total porosity is of fundamental importance for the growth of plants, since the high concentration of roots formed in the containers requires a high supply of oxygen and rapid removal of the carbon dioxide formed.

As for microporosity, the substrates S1, S2 and S4 showed microporosity between 45 - 55% (Table 3), a range considered adequate according to the recommendation of Gonçalves and Poggiani (1996). Although the substrate S3 was above the range considered adequate, with 64.97% microporosity, it did not present any problems in the development of lettuce seedlings. Ramanathan and Alagesan (2012) when evaluating the efficiency of the conversion of organic flower residues by millipedes, attributed the highest humidity in millicomposts due to the compaction of fecal pellets when compared to composted residues without the presence of the millipedes.

As for macroporosity, Gonçalves and Poggiani (1996) consider the range of 35-45% to be the appropriate levels. However, all substrates showed macroporosity ranging from 28.31 to 30.17% (Table 3), considered average levels by the same authors. It can be observed that the macroporosity of the substrates S1, S2 and S3 (millicomposts) decreased as the processing time of these substrates was extended. The substrate S3, being obtained after 180 days, no longer presents the structures of fecal pellets as those observed in substrates S1 and S2, obtained after 90 and 125 days, respectively. This is due to the biological activity that is associated during the millicomposting process (isopods and microorganisms), which promotes the fragmentation of these structures, thus making the S3 substrate more pulverized, which results in lower levels of macropores in relation to substrates S1 and S2.

The water retention capacity of a substrate plays a fundamental role in supplying water to plants. Gonçalves and Poggiani (1996) consider values between 20 - 30 mL 50 cm^{-3} as adequate levels of water retention capacity. In this way, all substrates meet the levels established by these authors (Table 3), with the exception of S3 substrate, which was close to the range considered adequate (32.48 mL 50 cm^{-3}), due to relatively more microporous.

Variation of pH and electrical conductivity of substrates during the development of lettuce seedlings

The results of the analysis of variance are shown in Table 4, where it can be seen that for both pH and electrical conductivity there were highly significant effects of the treatments, seedling development time and treatment x time interactions.

At the beginning of the seedling development, the substrates formulated based on millicompost had pH values close to neutrality, and the SIPA substrate showed pH 8.34, which is slightly alkaline. Fig. 2 shows the results of weekly pH monitoring for the substrates used in the production during the 28 days of seedling development in the greenhouse and reveal the increase in pH values for all substrates from the seventh day. It can be observed that the pH increases in the substrate S3 surpassed the value obtained for the substrate S2 after 14 days, remaining in elevation until 21 days, when the substrates S1, S2 and S3 presented declines in their respective values of pH, differing from the substrate S4, which kept increasing throughout the period.

The changes in the pH values are due to changes in the excretion of H^+ which, in turn, are due to an imbalance in the absorption of cations and anions. Fig. 1 shows the fractions of N present in the substrates, and it is observed that the highest percentages of N-nitrate are present in the substrates S2, S3 and S4, thus explaining the increase in pH values.

Costa (2014) explains that the way nitrogen is supplied to plants ends up being partly responsible for the difference between the pH of the rhizosphere and that of the soil - in this case, the pH of the substrate. If nitrogen is supplied in the NH_4^+ form, it will generate a decrease in the rhizosphere pH, caused by the efflux of H^+ (H^+ outflow from the cell); however, if it is supplied in the form of NO_3^-, it will lead to an increase in the rhizosphere pH due to influx of H^+ (H^+ entry into the cell). Out of range pH values affect nutrient availability for cultivated plants. For substrates with pH values below 5.0, deficiencies of nitrogen, potassium, calcium, magnesium and boron may occur, whereas at pH values above 6.5 deficiencies of phosphorus, iron, manganese, zinc and copper are expected. (Kratz 2011). The substrate S1 showed pH 8.34 at 0 days (Fig. 2), hindering the development of lettuce seedlings, was observed from the seventh day, which resulted in lower quality seedlings 28 days after sowing (Fig. 4 and Table 5).

Ferraz et al. (2005) report that at pH 6.0 to 7.0, there is an adequate availability of nutrients in commercial mineral substrates, whereas for organic substrates this value varies from 5.2 to 5.5, with an ideal pH range from 5.5 to 6.5. The pH values were above the ideal range for all substrates, and for the substrates S2, S3 and S4, the pH ranged from 7.01 to 7.69 at 0 days (Fig. 2), neverthe-

Table 4 Analysis of variance for pH and electrical conductivity (EC) of the split plot design, with substrate as the main plot and time (days after sowing) as sub-plots, showing the significance levels and the coefficients of variation

Parameter	Significance level			CV%	
	Substrate	Days after sowing	Interaction	Plot	Subplot
pH	<0.001 **	<0.001 **	<0.001 **	3.23	1.70
EC	<0.001 **	<0.001 **	<0.001 **	29.29	24.66

**: significant at the 1.0% level

less these values were not able to cause damage in the development and quality of lettuce seedlings and corroborate with Costa (2014), who reports that lettuce is a species adapted to alkaline soils, tolerating pH from 6.5 to 7.5.

Possibly the higher pH at 0 days, which made the S1 substrate unfavorable in relation to the others, was the result of the lower presence of N-nitrate (Fig. 1) when compared to the substrates of the same process, S2 and S3. Leal et al. (2013) reported that the pH reduction in composting occurs due to acidification induced by the transformation of N-ammonium into N-nitrate, confirming the results presented here, where the less pH value can be observed in the substrates S2 and S3 at 0 days (Fig. 2), due to the increase in the levels of N-nitrate present in these substrates (Fig. 1).

The electrical conductivity (EC) values of the substrates ranged from 0.65 to 2.66 dS m^{-1}at 0 days (Fig. 3), the lowest value being recorded for the substrate S1 and the highest value for S4. The Fig. 3 shows the EC values measured weekly in the substrates, during the 28 days of seedling development in the greenhouse. It is possible to notice that there was a severe drop in the EC values of the S4 substrate until the seventh day. Although there was a drop in the values of the other substrates, it was lower when compared to the substrate S4, used as a control. From the 14th day onwards, there was an increase in the EC values in the substrates S1, S2 and S3 (millicomposts) with decreases after the 21st day, including for the substrate S4.

The electrical conductivity (EC) is indicative of the concentration of salts in the solution and provides a parameter for the estimate of salinity in the substrates. EC values between 2.0 to 4.0 dS m^{-1} are considered high for substrates, values from 1.0 to 2.0 dS m^{-1} are normal and less than 1.0 dS m^{-1} are considered low (Araújo Neto et al. 2009). Therefore, only the substrates S2 and S3 showed normal concentrations of salinity at 0 days (Fig. 2), while

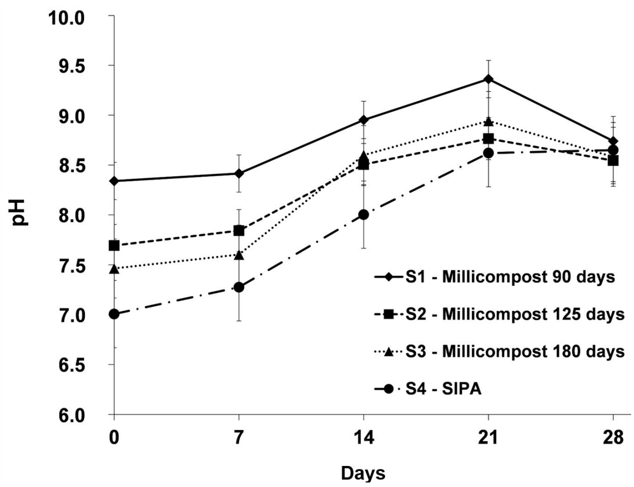

Fig. 2 pH values observed in the substrates during the development of curly lettuce seedlings cv. Vera. Average of four repetitions + standard error
SIPA substrate: 83% of vermicompost + 15% fine plant charcoal and 2% castor bean cake

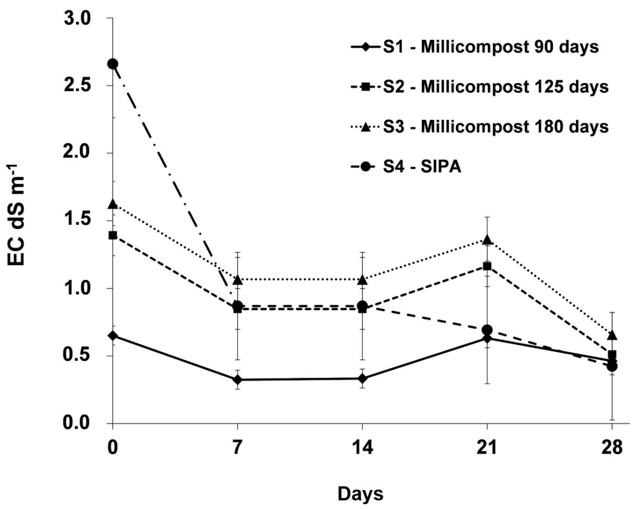

Fig. 3 Values of electrical conductivity (EC) observed in the substrates during the development of lettuce seedlings. Average of four repetitions + standard error
SIPA substrate: 83% of vermicompost + 15% fine plant charcoal and 2% castor bean cake

the substrate S4 showed high concentration, possibly due to the addition of castor cake in its formulation.

The substrate S1 presented low concentrations of salts, this was caused by the shorter processing time of the residues used in composting by the millipedes, resulting in lower values of macronutrients in relation to the substrates S2 and S3. The substrates S2 and S3, which were obtained at 125 and 180 days, respectively, show an increase in EC, a decrease in the C/N ratio and organic C, result of mineralization and availability of nutrients, promoted by the biological activity present in fecal and associative pellets. millicomposts (Table 2). Ramanathan and Alagesan (2012), evaluating the difference between vermicompost and millicompost in the production of pepper in pots, verified an increase of 27.40% in the EC of the millicompost with 60 days in relation to the 30 days, due to the increase in the nutritional levels of the millicompost, corroborating with the results presented here.

This weekly monitoring generated relevant information about the nutrient retention capacity in these substrates, which, in the face of daily irrigation, may undergo leaching. As previously mentioned, organic substrates have N mobilization and mineralization cycles due to the biological activity present in them. Possibly the rapid mineralization of the castor cake added to the S4 substrate and subsequent leaching due to the daily irrigation levels caused this decrease in the EC from 2.66 to 0.87 dS m^{-1} seven days after sowing (Fig. 3).

The decreases in the EC values for all substrates after 21 days probably occurred due to the nutritional demand by the lettuce seedlings, which were approaching the time of transplantation to the production beds. Thus, even though all substrates are of organic origin, the substrates S1, S2 and S3 (millicomposts) were able to maintain the levels of electrical conductivity more stable in relation to the substrate S4, that is, they were able to retain and provide nutrients to the lettuce seedlings by gradually releasing them.

Development of lettuce seedlings

There were significant differences in all parameters evaluated in this study. Only the result of the fresh mass of the aboveground plant (FMAP) of the seedlings produced on substrate S1 differed statistically and was lower than the other substrates (Fig. 4). The results of dry mass of the aboveground plant (DMAP) followed the same trend as FMAP (Fig. 4). S1 and S2 had significantly smaller root fresh (FMR) and dry mass (DMR, Fig. 4). Even though not statistically significant, FMR was 23.5 percent smaller for substrate S3 compared to S4.

The substrates are of fundamental importance for the performance of vegetable seedlings, since their chemical and physical characteristics are capable of directly influencing the fresh and dry mass of the aboveground plant and roots and at the end of the seedling phase, they provide the obtaining healthy and vigorous plants, which is desirable. Furlan et al. (2007) observed the best formation of cabbage seedlings in alternative substrates when compared to commercial substrates, with greater accumulation of dry mass of the aboveground plant and dry mass of the roots, highlighting the greater efficiency of mixtures of vermicompost, carbonized rice husk and rock dust as a substrate, providing greater growth.

Ramanathan and Alagesan (2012) compared peppers in pots with substrates based on vermicompost and millicompost generated at 60 days, and obtained the best parameters of plant height, number of leaves, leaf area, number and weight of fruits in the millicompost. Although all the substrates used in this work are organic, the millicomposting processing time ended up influencing the final availability of nutrients, which resulted in lower seedling performance for the S1 substrate (Fig. 4 and Table 5).

The variations in the weight of fresh and dry root mass can be attributed to the physical and chemical characteristics of the substrates, which interfered in the root development of the lettuce seedlings during the cultivation phase. According to Santos and Carlesso (1988), the water deficit is able to stimulate the expansion of the root system in search of more humid areas. Possibly, the lower water retention capacity of substrate S4 (Table 2) stimulated root expansion, providing this difference. Ferraz et al. (2005) reported that the substrate has a marked influence on the root system, especially with regard to the quantity and size of the particles, which define the aeration and water retention necessary for root growth. Thus, the lower weight of the fresh root mass on the substrate S3 is justified by the fact that it has a water holding capacity 34.90% higher than the substrate S4 (Table 2). However, the dry root mass on the S3 substrate showed a higher average value when compared to all other substrates (Fig. 4).

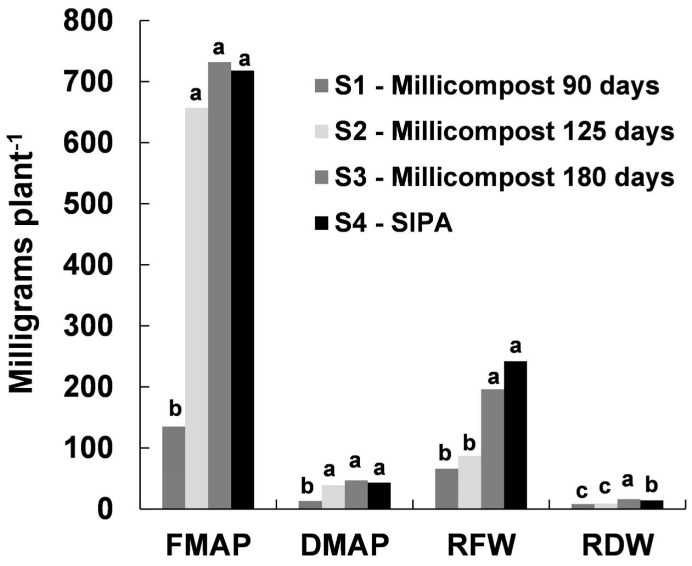

Fig. 4 Average values from fresh mass of the aboveground plant (FMAP), dry mass of the aboveground plant (DMAP), fresh mass of the roots (FMR) and dry mass of the roots (DMR) of curly lettuce cv. Vera produced on four different substrates, at 28 days after sowing
Means followed by the bar letter in the column do not differ by the Scott-Knott test (≤0.05)
SIPA substrate: 83% of vermicompost + 15% fine plant charcoal and 2% castor bean cake

The S1 substrate showed statistically lower values than the other substrates for the number of leaves, plant height, seedling vigor and clod stability (Table 5), attributing these results to the chemical characteristics of this substrate (Table 2). The substrates S2, S3 and S4 followed the same trend in the average values of number of leaves and seedling vigor, being only the height of plant higher for the substrate S3 in relation to the substrates S4 and S2, although they did not present statistical differences. It is possible to notice that the best stability of the clod was provided by the substrates S3 and S4 (Table 5), which differed statistically between themselves and the others. The substrates S1 and S2 were similar and did not differ from each other; however, they were inferior to the substrates S3 and S4, with clod stability notes close to 1, which resulted in 50% or more of the clod retained in the container when seedlings were removed.

The lower seedling vigor, for the S1 substrate seedlings, can be attributed to the chemical characteristics of this substrate, whose nutrients levels were lower than the other tested substrates (Table 2) and to its physical characteristics, where the particles of this substrate still preserved the structure of the fecal pellets of the millipedes, which may have provided a lower root system adhesion on the substrates S1 and S2, making the clod stability lower when compared to the clod stability of the S3 and S4 substrates (Table 5). Although substrate S2 showed less root stability, it was able to provide seedling development with similar vigor to the substrates S3 and S4, demonstrating its excellent capacity in the supply of nutrients (Table 2), water and aeration to the seedlings (Table 2). For transplanting in the field, the seedlings that combine better vigor and stability of the clod will end up reflecting on the formation of uniform stands and provide high survival rate, reducing or eliminating the need for seedling replanting.

The use of organic waste as substrates for vegetable production has been successfully confirmed in recent years and the use of millicomposts as an organic sub-

Table 5 Number of leaves (NL), plant height in centimeters (PH), seedling vigor (SV), clod stability (CS) and coefficient of variation (CV) of curly lettuce seedlings cv. Vera, grown on four different substrates, at 28 days after sowing

Substrates	NL	PH(cm)	SV	CS
Millicompost 90 days	3.28 b	3.25 b	3.38 a	1.43 c
Millicompost 125 days	4.75 a	6.87 a	1.08 b	1.33 c
Millicompost 180 days	4.48 a	8.62 a	1.23 b	3.03 b
SIPA	4.60 a	7.63 a	1.12 b	3.77 a
CV (%)	6.29	23.41	18.02	17.65

Means followed by the same letter in the column do not differ by the Scott-Knott test (≤0.05)
SIPA substrate: 83% of vermicompost + 15% fine plant charcoal and 2% castor bean cake.

strate and of low cost corroborates with other studies, such as that of Medeiros et al. (2007), who evaluated different substrates in the production of lettuce seedlings and observed that the substrate based on organic compost was the one with the highest values for all evaluated phytotechnics characteristics and by Simões et al. (2015), who tested different substrate conditioners, recording relevant results in the production of lettuce with organic compost.

According to Pereira (2013), the use of organic composts as substrates propitiates the development of more vigorous seedlings, for the reason that they are capable of providing the necessary nutrients for growth for various crops and currently represent an alternative to reduce the cost of production of vegetable seedlings (Silva Júnior et al. 2014).

Conclusion

The millicompost obtained at 90 days showed lower nutrient (N, Ca, Mg, P, and K) content, compared to the 125- or 180-day millicompost. The seedlings grown in the 180-day millicompost had a greater accumulation of biomass, greater height, and clod stability, that is, leaving smaller amounts of substrate in the cells when the seedlings were removed for evaluation. These results confirm the viability of using millicompost as a substrate to produce good quality lettuce seedlings from using a mixture of various plant residues with cardboard.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

References

Ambarish CN, Sridhar KR (2013) Production and quality of pill-millipede manure: A microcosmo study. Agric Res 2: 258-264. https://doi.org/10.1007/s40003-013-0075-5

Antunes LFS, Scoriza FN, Silva DG, Fernandes MEF (2016) Production and efficiency of organic compost generated by millipede activity. Ciênc Rural 46: 815-819. http://dx.doi.org/10.1590/0103-8478cr20150714

Antunes LFS, Scoriza RN, França EM, Silva DG, Correia MEF, Leal MAA, Rouws JRC (2018) Desempenho agronômico da alface crespa a partir de mudas produzidas com gongocomposto. Rev Bras Agropecu Sustent 8: 57-65. https://doi.org/10.30945/rcr-v21i2.2698

Antunes LFS, Scoriza RN, Silva DG, Correia MEF (2019) Consumo de resíduos agrícolas e urbanos pelo diplópode *Trigoniulus corallinus*. Nativa 7: 162-168. http://dx.doi.org/10.31413/nativa.v7i2.6192

Aquino AM, Almeida DL, Silva VF (1992) Utilização de minhocas na estabilização de resíduos. Comunicado Técnico n. 8. Embrapa Agrobiologia

Araújo Neto SE, Azevedo JMA, Galvão RO, Oliveira EBL, Ferreira RLF (2009) Produção de muda orgânica de pimentão com diferentes substratos. Ciênc rural 39: 1408-1413. http://dx.doi.org/10.1590/S0103-84782009005000099

Bhering LL (2017) Rbio: A tool for biometric and statistical analysis using the R platform. Crop Breed 17: 187-190. http:// dx.doi.org/10.1590/1984-70332017v17n2s29

Bianchi MO, Correia MEF (2007) Mensuração do consumo de material vegetal depositado sobre o solo por diplópodes. Seropédica: Embrapa Agrobiologia 4p. Technical Circular 20

Brasil (2008) Ministério da Agricultura, Pecuária e abastecimento (MAPA). Secretaria de Defesa Agropecuária (SDA). Instrução Normativa SDA Nº 31 de 23 de outubro de 2008. Altera os subitens 3.1.2, 4.1 e 4.1.2, do Anexo da Instrução Normativa SDA nº 17, de 21 de maio 2007. Métodos Analíticos Oficiais para Análise de Substratos para Plantas e Condicionadores de Solo. Diário Oficial da União, Brasília, DF, 24 de dez. 2008

Campanharo M, Rodrigues JJV, Lira Junior MA, Espíndola MC, Costa JVT (2006) Características físicas de diferentes substratos para produção de mudas de tomateiro. Caatinga. 19:140-145

Carrijo OA, Liz RS, Makishima N (2002) Fibra da casca do coco verde como substrato agrícola. Hortic Bras 20: 533-535. http://dx.doi.org/10.1590/S0102-05362002000400003

Costa AR (2014) Nutrição Mineral de Plantas Vasculares. Escola de Ciências e Tecnologia da Universidade de Évora, Portugal

Costa E, Santo TLE, Silva AP, Silva LE, Oliveira LC, Benett CGS, Benett KSS (2015) Ambientes e substratos na formação de mudas e produção de frutos de cultivares de tomate cereja. Hortic Bras 33:110-118. http://dx.doi.org/10.1590/S0102-053620150000100018

Cotta JAO, Carvalho NLC, Brum TS, Rezende MOO (2015) Compostagem versus vermicompostagem: Comparação das técnicas utilizando resíduos vegetais, esterco bovino e serragem. Eng Sanit Ambient 20:65-78.

https://doi.org/10.1590/S1413-41522015020000111864

Da Ros CO, Rex FE, Ribeiro IR, Kafer PS, Rodrigues AC, Silva RF, Somavilla L (2015) Uso de Substrato Compostado na Produção de Mudas de *Eucalyptus dunnii* e *Cordia trichotoma*. Floram 22: 549-558. http://dx.doi.org/10.1590/2179-8087.115714

Dores-Silva PR, Landgraf MD, Rezende MOO (2013) Processo de estabilização de resíduos orgânicos: vermicompostagem versus compostagem. Quím. Nova 36: 640-645. http://dx.doi.org/10.1590/S0100-40422013000500005

Fermino MH (2002) O uso da análise física na avaliação da qualidade de componentes e substratos. In: Furlani AMC. et al. (Coord). Caracterização, manejo e qualidade de substratos para produção de plantas. 1.ed. Campinas: Instituto Agronômico. p. 79

Fermino MH (2003) Métodos de análise para caracterização física de substratos para plantas. 81f. Ph.D. Thesis – Faculty of Agronomy, Federal University of Rio Grande do Sul, Porto Alegre

Fernandes C, Corá JE, Braz LT (2006) Desempenho de substratos no cultivo do tomateiro do grupo cereja. Hortic. Bras., 24: 42-46. https://doi.org/10.1590/S0102-05362006000100009

Ferraz MV, Centurion JF, Beutler AM (2005) Caracterização física e química de alguns substratos comerciais. Acta Sci Agron 27: 209-214. https://doi.org/10.4025/actasciagron.v27i2.1483

Franzin SM, Menezes NL, Garcia DC, Santos OS (2005) Efeito da qualidade das sementes sobre a formação de mudas de alface. Hortic Bras 23: 193-197. http://dx.doi.org/10.1590/S0102-05362005000200006

Furlan F, Costa M, Costa LA, Marini D, Castoldi, Souza J, Pivetta L (2007) Substratos alternativos para produção de mudas de couve em sistema orgânico. RBA 2: 1689

Garg VK, Yaday A (2011) Vermicomposting: An effective tool for the management of invasive weed Parthenium hysterophorus. Bioresour Technol 102: 5891-5895. https://doi.org/10.1016/j.biortech.2011.02.062

Gonçalves JLM, Poggiani F (1996) Substrato para produção de mudas florestais. In: Solo-Suelo- Congresso Latino Americano de Ciência do Solo, 13, 1996. Águas de Lindóia-SP. Relação de trabalhos. Águas de Lindóia: List of works. Lindoia Waters: Sociedade Brasileira de Ciência do Solo

Gruszynski C (2002) Resíduo agro-industrial “casca de tungue” como componente de substrato para plantas. Ph.D. Thesis. Porto Alegre: UFRGS

Kämpf NA (2005) Seleção de materiais para uso como substrato. In: Kämpf AN, Fermino M H (Ed.). Substratos para plantas: A base da produção vegetal em recipientes. Porto Alegre Gênesis: 139-145

Kania G, Klapec T (2012) Seasonal activity of millipedes (Diplopoda) - their economic and medical significance. Ann Agr Env Med 19: 646-650

Karthigeyan M, Alagesan P (2011) Millipede composting: A novel method for organic waste recycling. Recent Res Sci 3: 62-67

Kempers AJ, Zweers A (1986) Ammonium determination in soil extracts by the salicylate method. Commun Soil Sci Plant Anal 17: 715-723. https://doi.org/10.1080/00103628609367745

Kratz D (2011) Substratos renováveis na produção de mudas de *Eucalyptus benthamii* Maiden et Cambage e *Mimosa scabrella* Benth. MSc Dissertation. Curitiba: Universidade Federal do Paraná

Lea MAA, Guerra JGM, Espindola JAA, Araújo ES (2013) Compostagem de misturas de capim-elefante e torta de mamona com diferentes relações C: N. Rev. bras. eng. agríc. ambient. 17: 1195-1200. https://doi.org/10.1590/S1415-43662013001100010

Medeiros DC, Lima BAB, Barbosa MR, Anjos RSB, Borges RD, Cavalcante Neto JG, Marques LF (2007) Produção de mudas de alface com biofertilizantes e substratos. Hortic Bras 25: 433-436. http://dx.doi.org/10.1590/S0102-05362007000300021

Ministry of Agriculture, Livestock and Supply (MAPA) (2009) Agricultural Defense Secretariat. Normative Instruction no. 25. 23 July 2009. Official Gazette of the Federative Republic of Brazil. Executive power, Brasília

Miyazawa M, Pavan MA, Block MFM (1985) Spectrophotometry determination of nitrate in soil extracts without chemical reduction. Pesq Agrop Bras 20: 129-133

Nelson DW, Sommers LE (1996) Total carbon, organic carbon, and organic matter. In: Sparks DL. et al (Eds.). Methods of Soil Analysis. Madison: SSSA and ASA. 983 p

Oliveira EAG, Ribeiro RLD, Guerra JGM, Leal MAA, Espíndola JAA, Araújo ES (2011) Substrato produzido a partir de fontes renováveis para a produção orgânica de mudas de hortaliças. Seropédica: Embrapa Agrobiologia, (Boletim técnico), 4P

Oliveira JR, Xavier FB, Duarte NF (2013) Húmus de minhoca associado a composto orgânico para a produção de mudas de tomate. Agrogeoambiental 5: 79-86. http://dx.doi.org/10.18406/2316-1817v5n22013508

Oliveira Júnior JFO, Delgado RC, Gois G, Lannes A, Dias FO, Souza JC, Souza M (2014) Análise da precipitação e sua relação com sistemas meteorológicos em Seropédica, Rio de Janeiro. Floram 21: 140–149. http://dx.doi.org/10.4322/floram.2014.030

Olsen KK (2008) Multiple wavelength ultraviolet determinations of nitrate concentration, method comparisons from the preakness brook monitoring project, October 2005 to October 2006. Water Air Soil Pollut 187: 195–202. https://doi.org/10.1007/s11270-007-9508-8

Pereira MS (2013) Mineralização do resíduo da pupunheira em condições de campo e laboratório. MSc Dissertation. Viçosa: Universidade Federal de Viçosa

Ramanathan B, Alagesan P (2012) Evaluation of millicompost versus vermicompost. Curr Sci 103: 140-143

Santos RF, Carlesso R (1998) Déficit hídrico e os processos morfológico e fisiológico das plantas. Agriambi 2: 287-294. http://dx.doi.org/10.1590/1807-1929/agriambi.v2n3p287-294

Schmitz JAK, Souza PVD, Kämpf NA (2002) Propriedades químicas e físicas de substratos de origem mineral e orgânica para o cultivo de mudas em recipientes. Cienc Rural 32: 973-944. http://dx.doi.org/10.1590/S0103-84782002000600005

Shelley RM, Carmany RM, Burgess J (2006) Introduction of the milliped, *Trigoniulus corallinus* (Gervais 1847) (Spirobolida: Trigoniulidae), in Florida, U.S.A. Entomol News 117: 239-241.

https://dx.doi.org/10.3157/0013-872X(2006)117[239:IOT-MTC]2.0.CO;2

Silva LJB, Cavalcante ASS, Araújo Neto SE (2009) Produção de mudas de rúcula em bandejas com substrato a base de resíduos orgânicos. Cienc Agrotec 33: 1301-1306. http://dx.doi.org/10.1590/S1413-70542009000500015

Silva Júnior JV, Beckmann-Cavalcante MZ, Brito LPS, Avelino RC, Cavalcante IHL (2014) Aproveitamento de materiais alternativos na produção de mudas de tomateiro sob adubação foliar. Rev Cienc Agron 45: 528–536. http://dx.doi.org/10.1590/S1806-66902014000300013

Simões AC, Alves GKEB, Ferreira RLF, Araujo Neto SE (2015) Qualidade da muda e produtividade de alface orgânica com condicionadores de substrato. Hortic Bras 33: 521-526. http://dx.doi.org/10.1590/S0102-053620150000400019

Souza EGF, Barros Júnior AP, Silveira LM, Santos MG, Silva EF (2013) Emergência e desenvolvimento de mudas de tomate IPA 6 em substratos. Contendo esterco ovino. Ceres 60: 902-907. http://dx.doi.org/10.1590/S0034-737X2013000600020

Steffen GPK, Antoniolli ZI, Steffen RB, Machado RG (2010) Casca de arroz e esterco bovino como substratos para a multiplicação de minhocas e produção de mudas de tomate e alface. Acta Zool Mex 2: 333-343. http://dx.doi.org/10.21829/azm.2010.262898

Taíz L, Zieger E (2004) Plant physiology, 3rd edition, Porto Alegre, 719p. (In Portuguese)

Teixeira PC, Donagema GK, Fontana A, Teixeira WG (2017) Manual de métodos de análise do solo. Brasília: Embrapa

Thakur PC, Apurva P, Sinha SK (2011) Comparative study of characteristics of biocompost produced by millipedes and earthworms. Adv Appl Sci Res 2: 94-98