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Crambe wastes vermicomposting in arugula seedlings production

Vermicompostagem de resíduos de crambe na produção de mudas de rúcula

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ABSTRACT

Crambe is a plant with high potential for biodiesel production. However, its use for animal nutrition poses toxicity. The aim of this study was to propose an alternative treatment for this type of agro-industrial waste. Thus, we used earthworm *Eisenia foetida* for the stabilization of crambe residue. Twenty vermireactors were constructed, receiving crambe waste and cattle manure in the dose according to the determination of total solids. Treatments were called 100% of cattle manure; 10% waste crambe + 90% cattle manure; 20% waste crambe + 80% cattle manure; 30% waste crambe + 70% cattle manure; 40% waste crambe + 60% cattle manure. Mixtures remained for 40 days in vermireactors. The statistical design was completely randomized, with five treatments and four replications. The vermicompost obtained was used to produce arugula seedlings. At the end of the proposed period, it was observed that the worms survived in all crambe residue concentrations. Furthermore, it was found that even with the highest dose, crambe residue was not harmful worms. In the production of arugula seedlings, it was found that there was adequate development of plants.

Keywords: Agro-industrial waste, *Crambe abyssinica* Hochst, *Eisenia foetida*.

RESUMO

O crambe é uma planta com alto potencial de produção biodiesel. Entretanto, o uso para alimentação animal apresenta toxicidade. O objetivo do presente estudo foi propor uma alternativa de tratamento para este tipo de resíduos agro-industrial. Desta forma, utilizou-se a minhoca *Eisenia foetida* para a estabilização do resíduo de crambe. Foram construidos 20 vermireatores, que receberam resíduos de crambe e esterco bovino nas dose de acordo com a determinação de sólidos totais. Os tratamentos foram chamados 100% de esterco bovino; 10% resíduo de crambe + 90% esterco bovino; 20% resíduo de crambe + 80% esterco bovino; 30% resíduo de crambe + 70% esterco bovino; resíduo de crambe 40% + 60% esterco bovino. As misturas permaneceram durante 40 dias nos vermireatores. O delineamento estatítico foi inteiramente casualizado, com cinco tratamento e quatro repetições. Os vermicompostos obtidos foram utilizados na produção de mudas de rúculas. No final do período proposto, observou-se que as minhocas sobreviveram em todas as concentrações de resíduos de crambe. Além disso, verificou-se que mesmo com as doses mais elevadas do resíduo de crambe, não foi prejudicial as minhocas. Na produção de mudas de rúcula, verificou-se que houve um desenvolvimento adequado das plantas.

Palavras-chave: *Crambe abyssinica* Hochst, *Eisenia foetida*, resíduos agroindustriais.

INTRODUCTION

Renewable fuels demand has considerably grown in recent years, quickly expanding. This interest is due to several factors, such as local agriculture encouragement, oil-derived energy dependence reduction (Lobô *et al*., 2009), and concerns about the environment (Brasil, 2012).

Among oilseeds that stand out for biodiesel production in Brazil, soybeans are the first, reaching 90% of Brazilian vegetable oils production, followed by oil palm, coconut and sunflower (Mapa, 2005). However, due to these oilseeds added value, alternative low value raw materials are sought for biodiesel generation (Barros *et al*., 2006).

Considering oil content, yield, production system, and crop cycle aspects, crambe (*Crambe abyssinica* Hochst) has great potential to constitute biodiesel production raw material (Jasper *et al*., 2010). Its seeds have oil percentages ranging from 35 to 38%, which are exclusively for industrial use, as it is non-edible (Embrapa, 2012). Moreover, crambe oil is distinguished from other vegetable oils by its high erucic acid content, between 50 and 60% (Falasca *et al*., 2009).

Despite the advantages offered by crambe crop in biodiesel production, oil extraction generates crambe wastes, which does not have an appropriate destination, as it is toxic as animal feed (Pitol *et al*., 2010; Gonçalves *et al*., 2013).

Therefore, vermicomposting is shown as a way of treating organic residues. This technique makes use of epigeal worms to promote stabilization of organic materials (Dores-Silva *et al*., 2013). Vermicomposting final product is called vermicompost, a high sustainable alternative, as it is a material rich in plant usable nutrients (Gómez-Brandón *et al*., 2013). In addition, it may be also used as seedling production substrate, provided that it is within safe use limits (Sinha *et al*., 2010).

Given the need for crambe waste management and its proper treatment, the aim of this study was to evaluate the vermicomposting process of cattle manure with crambe wastes addition. In addition, vermicompost agronomic potential in arugula seedlings (*Eruca sativa* L.) development was also assessed.

MATERIAL AND METHODS

The experiment was conducted at Dinâmica das Cataratas University Center – UDC, located in the city of Foz do Iguaçu, Paraná, Brazil. In July 2014, 40 kg fresh cattle manure was collected in a farm in the city of Hernandarias, Paraguay. After collection, a cattle manure pile was made, followed by pre-composting for 30 days. During this period, manure was irrigated and plowed every two days, in order to aerate the medium.

Crambe wastes (30 kg) was obtained by cold pressing, which had approximately 15% oil. The collection site was the city of Cascavel, Paraná State, Brazil, in July 2014.

Eisenia foetida earthworms used in the experiment were donated by Paraguay Itaipu Binational forest nursery.

20 wooden boxes (vermireactors) were built for the vermicomposting process. The boxes were put horizontally, and the bottom was coated with shading cloth for air exchange, with 18,4 x 28 x 45 cm height, width, and length dimensions, respectively.

After thirty days of cattle manure pre-composting, this material was distributed in each vermireactor, with four crambe wastes doses, taking total solids determination into account. According to AOAC methodology (2005), 1 kg of total solids was used for each vermireactor. Treatments were named as follows: T_1: 100% cattle manure composition; T_1: 10% crambe wastes + 90% cattle manure; T_2: 20% crambe wastes + 80% cattle manure; T_3: 30% crambe wastes + 70% cattle manure. T_4: 40% crambe wastes + 60% cattle manure. Four replicates were carried out per treatment.

Ten earthworms were added in each vermireactor. They were first weighted, with the aid of a precision scale, and then inserted into the corner of each vermireactor, in the source substrate, so that they could migrate to the new substrate. Vermireactors were randomized into two benches, following completely randomized design (CRD). Vermireactors byproduct received irrigation two to three times a week, or according to need. Straw was placed superficially in each vermireactor, in order to help substrate moisture and temperature control.

Worm development was evaluated weekly for forty days. At the end of the experiment, worms were removed from the vermireactors through sieving (Embrapa, 2011), and weighed on a precision balance again.

Total, fixed, and volatile solids, pH, and electrical conductivity determination were held on vermicomposts according to methodologies described by AOAC (2005). Carbon (C) was determined by muffle furnace ignition for 2 hours at 550 °C, according to methodology by AOAC (2005). For total Kjeldahl nitrogen quantification, sulfuric digestion was used (AOAC, 2005). For total N determination, Kjeldahl Microdestilador distillation was used (Mantovani *et al*., 2005). C/N ratio was calculated from carbon and nitrogen results in the sample.

For arugula (*Eruca sativa* L.) cultivation, 1 kg pots were used, and 10 seeds per pot were placed. T_0, T_1, T_2, T_3, and T_4 vermicomposts were tested, in CRD, with four repetitions, totaling 20 pots.

Pots were placed in a greenhouse, and irrigation was conducted twice a day, morning and evening. After germination (about 4 days), arugula plants development was accompanied by visual form, and thinning was held after 10 days, remaining 5 plants per pot. Selected parameters to check vermicompost quality in plant development in the different treatments were: shoot height, leaf number, leaf width, and fresh and dry matter.

Plant development parameter measurements were held in the University Center's Chemistry and Environmental Sanitation Laboratory (UDC). Plant height, leaf width, and leaf length linear dimension measurements were taken through a digital caliper. Precision scale was used in order to determine fresh matter. Afterwards, plants were placed in paper bags and brought to an oven, at 70 °C, in order to determine the dry matter. After 48 h, dry matter weighting on a precision scale proceeded.

Vermicomposting and arugula (*Eruca sativa*) seedlings development results interpretation were performed using statistical analysis, through ASSISTAT software, by Tukey test, at 5% probability.

RESULTS AND DISCUSSION

Vermicompost dry matter, moisture, volatile and fixed solids, pH and electrical conductivity were assessed. Analysis results are shown in Table 1.

Normative Instruction No. 25, July 23, 2009, from the ministry of livestock, farming, and supply – MAPA (Brasil, 2009), defines organic fertilizer quality standards and parameter limits. In addition, it describes classes A, B, C, and D, which correspond to raw materials used as worm food. According to MAPA criteria (Brasil, 2009), vermicomposts produced from crambe wastes, as being an agro-industry co-product, are a "B" class organic fertilizer. Maximum humidity is 50%, minimum pH is 6, and maximum C/N ratio is 14/1.

Regarding moisture, all treatments were above what was indicated by Brasil (2009), that is, above 50%. High humidity is probably justified due to the humus produced by earthworms being rich in organic matter. Therefore, it has high water retention capacity. In a study by Silva *et al*. (2011), when vermicomposting was applied in domestic sewage sludge with different soil concentrations, vermicomposting final humidity of 38,55% was obtained in the treatment with 100% domestic sludge; 32,19% in the treatment with 75% sludge and 25% soil; and 25,22% in the treatment with 50% sludge and 50% soil. It was observed that, in the extent to which soil concentration increased, moisture decreased. However, humidity increases in larger domestic sludge concentrations, as it retains water due to being rich in organic matter.

$T_{2,}$ followed by $T_{0,}$ showed the highest fixed solid concentrations, 48,47 and 48%, respectively. However, T_0 and T_1 have not statistically differed. Fixed solids indicate the mineral amount present in certain substances. According to Dores-Silva *et al*. (2013), in aerobic processes, such as vermicomposting, mineralization causes organic matter complexed carbon to be transformed into CO_2. Bernal *et al*. (2009) stated that, due to carbon release, other elements that were not lost in the process concentrate, valuing the generated product, that is, the vermicompost.

Valente *et al*. (2013) analyzed different commercial vermicomposts, in which fixed solid value variations of 75,3%, 30,2%, 71,5%, 69,7%, and 68,1% were found. In the present study, low fixed solid values were found in relation to results presented by Valente *et al*. (2013), which may mean that the compound was not mature. Hsu and Lo (1999) explained that fixed solids content is generally higher in mature vermicomposts.

Compost maturation is also determined by the C/N ratio. However, optimum value varies from one literature to another (Lacerda *et al*., 2012). Paullus *et al*. (2000) and Kiehl (2010) stated that the compost is stabilized when the C/N ratio is lower than 18/1. Brasil (2009) states that C/N maximum ratio should be of 14/1 for vermicompost sale. The obtained vermicompost C/N ratio showed higher values than those exposed by the authors mentioned above for maturation determination and compost stabilization.

Although the vermicomposting process took 40 days for byproducts stabilization in humus form, Embrapa (2005) states that the process may take 30 days or more, depending on used co-product type. Cattle manure composting, conducted over 30 days, showed no fermentation. Toxic gases are released and temperature is raised in this process, what could harm earthworms (Embrapa, 1996), since they have cutaneous respiration (Amabis and Martho, 2006). Thus, it was found that worms showed no difficulty migrating from their compost to the substrate prepared to be vermicomposted. Thus, it is confirmed that the compost was ideal for worms, and within the parameters defined by Lourenço (2010).

Regarding volatile solids, values did not exceed 55%, with little variation, as presented in the literature. Solid values, both fixed and volatile, vary according to byproduct characteristics. This can occur because, according to Dores-Silva *et al.* (2013), besides microbiological degradation process, there is the worm feeding process. Part of the matrix organic matter is used to their development in this process. Besides organic carbon incorporation, which is promoted by worms, there is the reverse process contribution, i.e., organic carbon mineralization to CO_2. Amorim *et al*. (2005) observed the effects on vermicomposting in different seasons, and obtained 68,4% as volatile solids final value, whose process was conducted in winter, with goat manure use.

In relation to pH, values were close to neutrality, in addition to being within the minimum value stipulated by Brasil (2009), of at least 6,0. Close to neutrality pH values suggest that vermicompost can have corrective action in the soil, since it can be incorporated therein (Soares *et al*., 2004). The pH results obtained by Valente *et al*. (2013) in a commercial vermicomposts study varied according to composition. Vermicompost 1 had 5,2 pH, vermicompost 2 had 7,4 pH, vermicompost 3 had 6,6 pH, vermicompost 4 had 6,0 pH, and vermicompost 5 had 5,7 pH. Thus, vermicomposts 1, 4, and 5 were acid, while vermicomposts 2, 3, and 4 were neutral.

During vermicomposting process, soluble salts increase can occur, i.e., vermicomposts electrical conductivity is increased. This is because worms

Table 1 - Crambe wastes vermicompost characterization at 40 days

Treatment	Dry Matter (%)	Humidity (%)	Fixed Solids (%)	Volatile Solids (%)	pH	CE ($mS.cm^{-1}$)	C/N
T_0	26,87 d	72,87 b	48,00 ab	51,35 d	6,35 e	1,30 c	30/1
T_1	27,50 c	72,30 b	47,57 b	52,37 c	6,48 d	1,82 a	27/1
T_2	25,65 e	74,85 a	48,47 a	51,42 d	7,06 a	1,40 bc	30/1
T_3	31,55 a	68,52 c	46,52 c	53,45 b	6,76 b	1,55 b	27/1
T_4	27,95 b	72,02 b	45,20 d	55,45 a	6,65 c	1,60 b	24/1
Dms	0,48	0,43	0,40	0,45	0,04	0,10	
CV (%)	7,32	2,95	2,64	3,04	3,76	13,24	

T_0: 100% cattle manure. T_1: 10% crambe wastes + 90% cattle manure. T_2: 20% crambe wastes + 80% cattle manure. T_3: 30% crambe wastes + 70% cattle manure. T_4: 40% crambe wastes + 60% cattle manure.

mineralize organic matter with the help of digestive tract microorganisms (Gómez-Brandón *et al*., 2013). In this study, conductivity values were between 1,30 to 1,60 $mS.cm^{-1}$, as demonstrated in the study of Lourenço (2010), who stated that values below 3 $mS.cm^{-1}$ are not harmful to earthworms.

Eisenia foetida was the assessed earthworm species, which was favorable to the co-products used in the vermicomposting process. This species, besides quickly reproducing, produces high vermicompost percentage, and is able to easily adapt in captivity (Martinez, 1995). Studies carried out by Kaur *et al*. (2010), Suthar (2012), and Dores-Silva *et al*. (2013), succeeded in keeping these worms in the vermireactors. However, in the study by Vig *et al*. (2011), the worms died.

After 40 days of vermicomposting, it was found that the crambe wastes had not adversely affected worms development, both in $T_{4,}$ with higher crambe wastes addition (40% wastes and 60% cattle manure) as in $T_{1,}$ with lower crambe wastes addition (10% wastes and 90% manure), having no earthworm mortality. Multiplication was found in 20 vermireactors, i.e., in all treatments, and their respective repetitions. From 20% crambe wastes, there was worm body mass increase (Table 2), indicating system viability.

Earthworms' initial weight matches the Fundación de Hogares Juveniles Campesinos (2005) statement, in which they have a weight of approximately 1 g, and are daily fed with organic matter equivalent to their weight. In the study by Suthar (2012), earthworms gained weight in the 7 treatments. However, the authors stated that body mass increase significantly varied between treatment 1, which consisted of 20% milk processing sludge and 80% cattle manure, and treatment 6, which consisted of 60% sludge and 40% straw. The authors reported that worms' body mass increase is related to compost palatability and particle size of the raw material used in the mixture. In addition, they also pointed out that plant residues have different appetence to mineral origin residues. The high protein and fiber content that these residues generally have can influence earthworms' growth.

Worm survival during crambe wastes treatment is an important fact to be highlighted, because, according to Pitol *et al*. (2010), crambe is rich in glucosinolates, besides having erucic acid, which are toxic substances. Thus, crambe use is not allowed in high percentages for ruminant animals feeding. The authors warned about damages caused to other types of animals, if crambe is consumed in lower percentages.

After vermicomposts stabilization, arugula (*Eruca sativa*) seeds planting were conducted. After 20 days of sowing, leaf width, leaf length, leaf number, plant height, and fresh and dry mass were assessed, which are shown in Table 3.

It was observed (Table 3) that the different crambe wastes doses used in vermicomposting treatments have not influenced arugula (*Eruca sativa* L.) seeds germination. 100% germination was obtained in all pots. Thus, it can be inferred that the vermicompost was favorable, even with crambe wastes inclusion, not statistically differing from the treatment without

Table 2 - Body mass average values of 10 worms on crambe wastes vermicomposting treatment

Treatments	Initial Mass (g)	Final Mass (g)
T_0	10,30 a	10,58 ab
T_1	10,40 a	10,05 b
T_2	10,20 a	12,60 ab
T_3	8,40 a	13,81 a
T_4	9,30 a	13,36 ab
Dms	6,50	3,58
CV (%)	30,70	15,57

Means followed by the same letter in the column do not differ by Tukey test at 5% significance. T_0: 100% cattle manure. T_1: 10% crambe wastes + 90% cattle manure. T_2: 20% crambe wastes + 80% cattle manure. T_3: 30% crambe wastes + 70% cattle manure. T_4: 40% crambe wastes + 60% cattle manure.

Table 3 - Vermicompost-grown arugula seedlings Shoot fresh matter, shoot dry matter, shoot dry matter content, root fresh matter, root dry matter, root dry matter content, plant height, leaf number, and leaf width

Treatments	Shoot Fresh Matter (g)	Shoot Dry Matter (g)	Dry matter content	Root Fresh Matter (g)	Root Dry Matter (g)	Dry Matter Content
T_0	0,57 a	0,040 a	70 %	0,0028 a	0,0020 a	72 %
T_1	0,59 a	0,036 a	61 %	0,0035 a	0,0020 a	57 %
T_2	0,52 a	0,029 a	55 %	0,0032 a	0,0023 a	72 %
T_3	0,46 a	0,030 a	65 %	0,0023 a	0,0020 a	70 %
T_4	0,54 a	0,039a	72 %	0,0061 a	0,0031 a	52 %
Dms	0,28	0,02		0,00	0,00	
CV (%)	23,86	30,03		49,48	125,79	

Treatments	Plant height (cm)	Leaf number	Leaf width (cm)
T_0	10,60 a	3,12 a	2,31 a
T_1	10,51 a	3,00 a	2,30 a
T_2	10,37 a	2,62 a	2,34 a
T_3	10,25 a	2,50 a	2,20 a
T_4	10,38 a	2,87 a	2,31 a
Dms	1,94	0,98	0,49
CV (%)	8,53	15,99	9,89

Means followed by the same letter in the column do not differ by Tukey test at 5% significance. T_0: 100% cattle manure. T_1: 10% crambe wastes + 90% cattle manure. T_2: 20% crambe wastes + 80% cattle manure. T_3: 30% crambe wastes + 70% cattle manure. T_4: 40% crambe wastes + 60% cattle manure.

crambe wastes. It is noteworthy that, even with the highest crambe dose (T_4), plants were developed, i.e., it was not toxic to inhibit their growth.

Thus, crambe wastes, crambe biodiesel production co-product, currently unusable or without economic value aggregation, can be used in arugula production vermicomposting as a sustainable practice for its use. The production of this crop in Brazil predominates in the South and Southeast regions of the country, where its development is favored by mild temperatures (Medeiros *et al*., 2007).

Oliveira *et al*. (2013) analyzed arugula seedlings development fertilized with vermicompost. The authors found that it had the necessary nutrients to arugula growth, without the need of chemical fertilizers use. According to Ferreira *et al.* (2012), this result can be attributed to the fact that the vermicompost produced by earthworms provides nutrients, such as magnesium and nitrogen, to the soil solution, which are part of the chlorophyll molecule.

CONCLUSION

Vermicomposts produced with crambe wastes insertion represent a technically feasible alternative for arugula seedlings production, making it possible to be produced on the farm where crambe residues are generated. Crambe wastes favors *Eisenia foetida* species mass gain. Therefore, vermicomposting is an alternative practice to crambe wastes allocation.

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