

DEVELOPMENT AND MULTIPLICATION OF *Eisenia andrei* IN THE MANURE OF CATTLE SUBJECTED TO HIGH TRICHODERMA DOSES

DESENVOLVIMENTO E MULTIPLICAÇÃO DE *Eisenia andrei* EM ESTERCO BOVINO EM ALTAS DOSES DE TRICHODERMA

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ABSTRACT: The efficacy of the vermicomposting and products based on the antagonistic fungus and plant growth promoter trichoderma (*Trichoderma* spp) is well known and studied in organic agriculture. However, for a better methodological efficiency are necessary studies to evaluate the effect of high doses of these bioproducts in the biology and development of earthworms. Thus, the present work aims to test the use of high commercial biocontrol product (ICB Nutrisolo Trichoderma) doses by evaluating the multiplication and development of *Eisenia andrei*. Changes in the chemical features of the substrate produced by the vermicomposting process using *in natura* and sterilized organic cattle manure were also assessed. Each experimental unit consisted of 6 kg of substrate (in multipurpose polypropylene box – 20 x 40 x 50 cm) containing 48 clitellate adult *Eisenia andrei* earthworms. ICB Nutrisolo Trichoderma was used as biological agent along with eight strains of the following species: *T. koningiopsis*, *T. asperellum* and *T. harzianum*. The following treatments were applied at doses of 10^{11} CFU kg⁻¹ of ICB Nutrisolo Trichoderma in the presence of earthworms: T1 (0.5); T2 (1.0); T3 (2.0); T4 (4.0); T5 (8.0) and T6 (0.0). The T7 treatment was herein used in order to evaluate the chemical features of the vermicompost. It was a completely randomized design with four replications per treatment. The temperature was kept at 28°C and humidity ranged between 60 and 70%. After 60 days, the number of young and adult earthworms, and cocoons was counted; then, their dry biomass was assessed. The results found in the lethality test showed decrease in the number of earthworms treated with 4.0×10^{11} CFU kg⁻¹ of ICB. The biological product doses up to 1.0×10^{11} CFU kg⁻¹ did not alter the number of adult earthworms and cocoons, or the multiplication index of *E. andrei* in cattle waste vermicomposts. There was no influence of the tested doses on earthworms' individual development. However, doses above 2.0×10^{11} CFU kg⁻¹ decreased their total biomass. The C/N ratio for all treatments indicates maturity within acceptable results for organic compounds.

KEYWORDS: *Trichoderma* spp.. Biological product. Worm. Vermicomposting.

INTRODUCTION

Vermicomposting - an organic waste decomposition process that uses earthworms - and trichoderma (*Trichoderma* spp.) are less aggressive to the environment; thus, they are alternatives used in organic agriculture to reduce the use of pesticides.

The efficacy of vermicomposting is directly related to the population, biology and reproduction of earthworms used in the decomposition of easily biodegradable organic matter, such as organic wastes from cattle, into humus. Dairy cattle breeding is one of the most important organic waste production sectors in Brazil (IBGE, 2009); however, the waste production volume in this system leads to soil, lake, river and groundwater contamination. Consequently, it is a serious environmental issue, which becomes a challenge for breeders and specialists (VAN HORN et al., 1994).

Studies have shown that vermicomposting, in comparison to compounds produced without

earthworms, accelerates organic matter stabilization and produces compounds with lower carbon-nitrogen (C/N) ratio, better cation exchange capacity, as well as with more humic substances (ALBANELL et al., 1988) and phytohormones (TOMATI et al., 1995). *Eisenia andrei* and *Eisenia fetida* are among the earthworm species most used to produce vermicomposts, since they are more efficient in transforming wastes, and because they present high multiplication index (DOMÍNGUEZ et al., 2010). In addition, they are tolerant to wide temperature and humidity ranges, besides being able to multiply in several waste types (ATIYEH et al., 2000); however, they do not tolerate acid wastes or wastes with very strong odor (OLIVEIRA et al., 2007).

Earthworms are environmental contamination bioindicators, because they are sensitive to volatile and non-volatile compounds, as well as to gases such as CO₂. Number of species, as well as abundance and biomass are easily measurable parameters in earthworms inhabiting

soils; thus, they are important tools to assess different environmental transformations and impacts. Parameters such as biomass and number of earthworms may be used to assess different cropping systems in rural environments (PAOLETTI, 1999).

Trichoderma is a promising phytopathogen-biocontrol agent, as well as a natural soil fungus mainly found in organic soils, which may saprophytically live or parasitize other fungi (MELO, 1996). Trichoderma isolates are cited as volatile and non-volatile metabolite producers (DENNI; WEBSTER, 1971a, b, c); among these metabolites, it is worth highlighting the volatile compound 6-pentyl- α -pyrone (6-PP), which is a lactone with strong coconut-like aroma that has fungicidal activity (COLLINS; HALIM, 1972; SERRANO-CARREÓN et al., 2004). According to Neumann and Laing (2006), trichoderma affects the normal nitrification process when it is added to plant roots at high doses and in the presence of ammonium ions, fact that leads to the risk of toxicity associated with plant growth inhibition. Oliveira (2008) conducted a bioremediation study in soil contaminated with petroleum and reported nitrogen-related toxicity causing high *E. andrei* lethality rate (100%).

Although studies have already shown the benefits from the organic agriculture by separately using earthworms and trichoderma, studies assessing the interaction between these organisms and the chemical properties of the soil remain scarce. According to Sadykova and Kurakov (2013), the combination between *Trichoderma asperellum* and earthworms was efficient in multiplying *E. fetida*, as well as in promoting growth and development of cucumber plants. Vermicomposting studies involving high trichoderma doses are not often found (NEUMANN; LAING, 2006); however, studies assessing high trichoderma dose applications associated with vermicomposting, as well as assessing changes in the chemical properties of humus, are essential to help improving application methodologies.

The current hypothesis is that high trichoderma doses affect earthworm development and change chemical properties in cattle manure. Thus, the aim of the present study was to test high doses of the commercial organic product ICB Nutrisolo Trichoderma by assessing *Eisinea andrei* multiplication and development, as well as by analyzing the changes in the chemical features of the substrate produced throughout the vermicomposting process by using organic wastes such as cattle manure.

MATERIAL AND METHODS

Adult and clitellate earthworms belonging to species *Eisenia andrei* were provided by the worm farm at Soils Department of Federal University of Santa Maria, Santa Maria County, RS. The commercial product ICB Nutrisolo Trichoderma was used in its liquid form as trichoderma biological agent source; the product was composed of 8 strains of *Trichoderma koningiopsis*, *Trichoderma asperellum* and *Trichoderma harzianum*, at concentration 10^{11} CFU mL⁻¹; MAPA registration n. RS12734/10000-4 (Article 15 of the Annex to Decree 4954/2004), and it was provided by ICB BIOAGRITEC LTDA.

Vermicompost production at high trichoderma doses

Increasing substrate doses higher than 10^6 CFU kg⁻¹, which was the dose described by Sadykova and Kurakov (2013) for *T. asperullum* in vermicomposting processes, were used to test the effect of high colony forming unit (CFU) concentrations of the commercial product ICB Nutrisolo Trichoderma (ICB) on earthworm reproduction and development in cattle manure. The substrate used to produce the vermicompost was dairy cattle manure *in natura*, which was obtained in a rural property located in Santa Maria County, RS. Treatments were set in 10 L capacity polypropylene multi-purpose boxes (dimensions 20 x 40 x 50 cm) added with 6 kg of substrate per repetition in each treatment, after the manure was homogenized and autoclaved twice at 121°C in a 24 hour interval. The assessed treatments were T1 (earthworms + 0.5×10^{11} CFU kg⁻¹ ICB), T2 (earthworms + 1.0×10^{11} CFU kg⁻¹ ICB), T3 (earthworms + 2.0×10^{11} CFU kg⁻¹ ICB), T4 (earthworms + 4.0×10^{11} CFU kg⁻¹ ICB), T5 (earthworms + 8.0×10^{11} CFU kg⁻¹ ICB) and T6 (earthworms and no ICB). The T7 treatment (no earthworms and no ICB) was used to assess the chemical features of the vermicompost at high ICB doses.

The experimental design was completely randomized with four repetitions per treatment. The commercial biological product ICB was applied to the substrate 7 days before earthworm inoculation in order allow colonization to take place. The boxes were covered with brown paper, the temperature was kept at 28°C, humidity ranged from 60% to 70%, and the entire process was conducted in the absence of light. The material was uniformly stirred every 7 days for aeration and humidity control purposes. Each experimental unit consisted of 48

adult clitellate earthworms belonging to species *E. andrei*.

The following assessments were carried out per experimental unit 60 days after earthworm inoculation: Manual count of the number of adult (clitellate), young (without developed clitellum), and total earthworms (adult and young), number of cocoons, ratio between the number of adult earthworms and cocoons, multiplication index, and total weight of young and adult earthworms.

The material from each experimental unit was placed on a white-background table to allow separating young from adult earthworms, as well as the cocoons found in the substrate. The collected individuals were separated in 100 mL plastic vials, where they were kept for approximately 8 hours, in lighted environment, to allow the material in their digestive tract to be eliminated. Subsequently, they were washed to remove wastes adhered to their bodies, dried with paper towel, and kept in an oven at 75°C, in open containers, until reaching constant dry weight. The earthworm multiplication index was calculated through the formula $MI = Pf / Pi$, wherein MI = multiplication index, Pf = final earthworm population and Pi = initial earthworm population, which corresponded to the number of inoculated matrices (STEFFEN, 2008).

Lethality test

The lethality test was conducted according to ISO 11268-1 - Soil quality - Effects of pollutants on earthworms (Part 1: Determination of acute toxicity using artificial soil substrate (ISO, 1993) - and according to ISO 11268-2 - Soil quality - Effects of pollutants on *E. fetida* earthworms (ISO, 1998) - in order to test the effect of the high ICB Nutrisolo Trichoderma doses (used in treatments T1, T2, T3, T4, T5 and control (T6)) on the number of surviving *E. andrei* individuals. The experimental unit consisted of a Styrofoam box (4 L) containing 3 kg of cattle manure *in natura* and 10 adult clitellate earthworms belonging to species *E. andrei*. Adult specimens were placed in each container, in the respective treatments, at the beginning of the lethality test. The number of living and dead earthworms was counted and compared to the control treatment (cattle manure, only) after 48 exposure hours in order to generate the survival curve.

Chemical featuring of the vermicompost

The total N, C, P, K, Ca and Mg rates, pH in the water, and C/N ratio were set according to the plant waste and tissue methodology by Tedesco et al. (1995) in order to assess the influence of

earthworms and trichoderma on the chemical quality of the substrate in T1, T2, T3, T4, T5, T6 and T7. The collected samples (100 g vermicompost) were dried in an oven at 75°C and ground in mortar, 60 days after inoculation, in order to be analyzed in the Tissue Analysis Laboratory.

Statistical analysis

Analysis of variance (ANOVA) was used to compare differences between treatments and, whenever necessary, the Scott-Knott test at 5% probability level was used to compare differences between means. Polynomial regressions were used to assess lethality, number of adult earthworms, number of cocoons, adult earthworms/cocoon ratio, and total earthworm weight. On the other hand, an exponential model was used to measure the effect of increasing trichoderma doses on the number of young earthworms, on the total number of earthworms and on earthworm multiplication index. All the analyses were performed in the BioEstat 5.0 (AYRES et al., 2007) and in the R software (R CORE TEAM, 2014).

RESULTS AND DISCUSSION

Results recorded in parameters such as number of adult and young earthworms, total number of earthworms, number of cocoons, adult earthworm/cocoon ratio, earthworm multiplication index and total earthworm dry weight showed that all treatments using high ICB doses did not lead to total mortality of individuals, as well as that they provided conditions for *E. andrei* reproduction. Some treatments showed reduction in all the aforementioned parameters when they were compared to the control without biological product (Figures 1 and 2).

The number of adult earthworms (Figure 1A) at ICB doses 0.5 and 1.0 (47.2) did not show significant difference in comparison to that of the ICB-free treatment (45.5); however, it differed from treatments using higher doses (2.0, 4.0 and 8.0 ICB), which showed gradual adult earthworm number decrease due to earthworm death or escape (34.2, 14.5, and 9.0, respectively). Higher ICB doses turned the environment unfavorable for earthworm adaptation and stabilization. According to Lavelle et al. (2004), the explanation for the earthworms' behavior and for the significant difference between results recorded in small-scale experiments, and in the real world, lies on the fact that confined earthworms have limited opportunities to find food and to move. It probably explains why they almost always lose weight or die in laboratory experiments.

According to the survival curve of *E. andrei* individuals (Figure 1F), the results recorded in the lethality test showed that the number of earthworms decreased only when doses equal to or higher than 4.0×10^{11} CFU kg^{-1} ICB were used. Results also

showed that the number of earthworms increased at the dose 0.5×10^{11} CFU kg^{-1} ICB, in comparison to the control (no ICB), thus corroborating the results presented in Figure 1A.

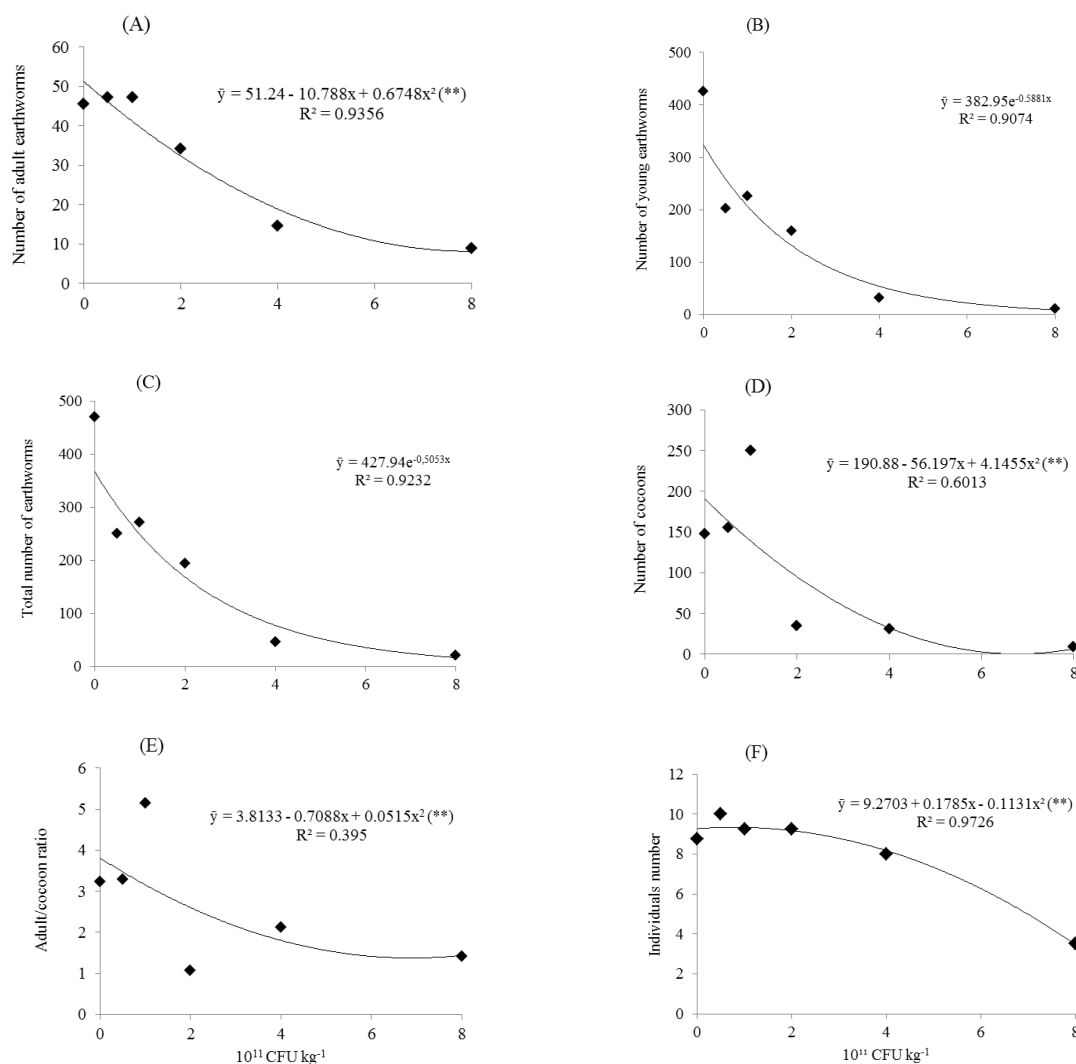


Figure 1. Number of adult (A) and young earthworms (B), total number of earthworms (C), number of cocoons (D), adult/cocoon ratio (E) and survival curve of *Eisenia andrei* individuals (F) under high ICB Nutrisolo Trichoderma doses. (**) significant at 0.01% probability level, according to the F test.

Studies investigating the interference of high trichoderma doses in the biology of organisms and in the chemical properties of humus are scarce; however, they are essential to help improving the bioproduct application methodology used in vermicomposting processes. According to Erickson (1985), although some toxicity can be attributed to ionized ammonia, the non-ionized form of it is acknowledged as the most toxic one. Oliveira (2008) conducted a study using contaminated soil added with urea at C/N ratio 100:10 and found high earthworm lethality (100%) in comparison to that of virgin soils (0% death), thus proving earthworm

sensitivity to these nitrogen sources. Reports associating high trichoderma doses with temporary growth delay in plants subjected to high temperature and ammonia concentrations have been found in the literature (NEUMANN; LAING, 2006). Ammonia is naturally found in water bodies as the product from soil degradation, as well as from the degradation of water organic and inorganic compounds, which result from biota excretion and from gaseous nitrogen reduction in the water. Such reduction is caused by microorganisms or by gas exchange with the atmosphere. Ammonia may be

found as ionized NH_4^+ or non-ionized NH_3 in aqueous solutions (REIS; MENDONÇA, 2009).

The multiplication index is the parameter used to assess the reproductive capacity of matrices in a given environment (ANTONIOLLI et al., 2009). The species *E. andrei* and *E. fetida* present similar features and behavior (ATIYEH et al., 2000). The multiplication index results recorded for *E. andrei* in treatments using ICB doses 0.5 (5.20), 1.0 (5.65) and 2.0 (4.02) were similar to those recorded by Aquino et al. (1994), who found *E. fetida* multiplication index 4.54 under similar conditions, as well as number of adult and young earthworms and cocoons (4.0, 18.5 and 1.5, respectively) smaller than that recorded in Figures 1A, B and D. The aforementioned authors pinpointed that, although cattle manure is a good food source for earthworms belonging to these species, its constitution may strongly vary depending on cattle diet, fact that makes it difficult comparing the experiments. Results recorded for the

first 3 doses (Figure 2A) used in the current study were also higher than those found by Pereira et al. (2005), who used pure cattle manure and recorded multiplication index 2.1.

Antoniolli et al. (2009) conducted a study with *E. fetida* and used mixtures comprising cattle manure and rice husk in the initial inoculation of 6 matrices in 4 L manure; they found higher multiplication index (8.5) and smaller numbers of cocoons (57) in the same period. Schiavon et al. (2007) conducted an experiment of similar nature and found a large number of cocoons in treatment using pure cattle manure; they inoculated 10 earthworms belonging to species *E. fetida* in 300 g substrate and collected 138 cocoons 28 days after inoculation, fact that showed *E. fetida* reproductive efficiency in this material. The sexual maturity of earthworms is related to the conditions of the environment they are in, and it results in greater or lesser multiplication within a certain time (AQUINO et al., 1994).

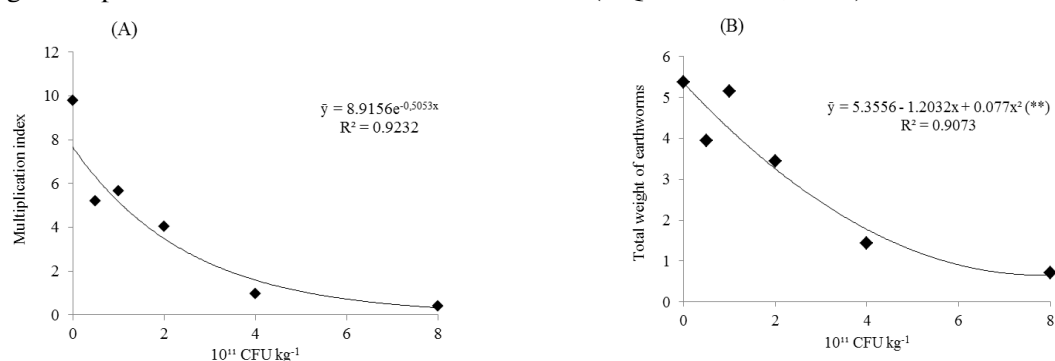


Figure 2. Multiplication index (A) and total weight of earthworms (B) under high ICB Nutrisolo *Trichoderma* doses. (***) significant at 0.01% probability level, according to the F test.

Treatments using high ICB doses such as 4.0×10^{11} CFU kg^{-1} and 8.0×10^{11} CFU kg^{-1} presented multiplication indices 0.96 and 0.41, respectively; these values were significantly lower than those resulting from the first 3 doses (Figure 2A). Sadykova and Kurakov (2013) used 10^6 CFU kg^{-1} of *Trichoderma asperellum* MG 97 isolate in vermicompost produced from manure, plant remains and sawdust. They found that antagonist addition, at the lowest dose, enabled an efficient way to recycle organic wastes with increased phytopathogen suppressive effect and growth-promoting effect on plants, as well as with increased earthworm production and proportion of mature individuals in the population. The referred dose used by the aforementioned author was much lower than the lowest dose used in the current study (0.5×10^{11} CFU kg^{-1} of ICB Nutrisolo *Trichoderma* in 6 kg vermicompost derived from cattle manure). These

higher doses may have decreased the number of individuals in the earthworm population susceptible to volatile and non-volatile compounds, which can be produced by trichoderma isolates (DENNIS; WEBSTER, 1971a, b, c).

Gases such as ethylene and hydrogen cyanide (CAMPBELL, 1989), as well as acetaldehyde, acetone, ethanol and carbon dioxide (TAMIMI; HUTCHINSON, 1975), are among the volatile metabolites produced by trichoderma. Earthworms are sensitive to such compounds and gases; thus, they are considered environmental contamination bioindicators. The release of these toxic substances may be related to the high doses of the biological product that were in contact with the organic matter, in the present case, cattle manure.

Studies about the effects of volatile, non-volatile substances and gases produced by trichoderma on earthworm development and

multiplication are scarce; however, it was already reported that trichoderma affects the nitrification process and may lead to toxicity when it is used at high doses in the presence of ammonium ions (NEUMANN; LAING, 2006). Some trichoderma isolates produce the volatile compound 6-pentyl- α -pyrone (6-PP), which is a lactone presenting biocidal activity (COLLINS; HALIM, 1972; SERRANO-CARREÓN ET AL., 2004). According to Manganot and Diem (1979), CO₂ is one of the most studied volatile substances produced by antagonists and its effects on fungi are quite varying, i.e., these effects can be stimulating or inhibitory, as it happens with ammonia. Ethylene enables the formation of inhibitory derivatives such as allyl alcohol. Metabolite production by soil antagonists is not well understood; thus, it is strongly based on assumptions (CLAYDON ET AL., 1987).

There was total earthworm dry weight (Figure 2B) variation between treatments according to the herein tested ICB doses; doses higher than 2.0

led to decrease in the total dry weight of *E. andrei*. Such decrease is directly related to the multiplication index and to the number of earthworms presented in Figures 2A, 1A, 1B, 1C, respectively. Schirmer (2010) did not find significant difference in the parameter 'weight of adult individuals' subjected to sewage sludge and cattle manure at 2 different ratios - (3/1) and (1/1).

Parameters such as pH, as well as P, K and Mg rates showed variation; however, C, N and Ca rates did not show significant differences between treatments (Table 1). The pH values did not vary between ICB treatments and were within the range found in materials of similar nature (PEQUENO ET AL., 2008; VIDAL ET AL., 2007; ANTONIOLLI ET AL., 2002). In addition, pH or temperature increases shift the chemical balance towards non-ionized ammonia (REIS; MENDONÇA, 2009). However, Soares et al. (2004) stated that pH values close to neutrality suggest that the vermicompost may present acidity-correction action when it is incorporated to the soil, since it shows buffering property.

Table 1. Chemical analysis applied to pH in the water, C/N ratio, and total N, C, P, K, Ca and Mg rates in vermicompost from cattle manure, at high ICB Nutrisolo Trichoderma doses.

ICB Doses*	pH water 1/1	C (%)	N (%)	C/N	P (%)	K (%)	Ca (%)	Mg (%)
0.5	7.86 a	26.3 a	1.90 a	13/1	2.95 b	0.08 a	1.82 a	0.60 a
1.0	7.94 a	26.7 a	2.07 a	12/1	2.90 b	0.08 a	1.78 a	0.60 a
2.0	7.88 a	24.5 a	2.70 a	9/1	2.91 b	0.08 a	1.96 a	0.63 a
4.0	7.92 a	27.0 a	2.16 a	12/1	3.38 a	0.09 a	2.24 a	0.62 a
8.0	7.91 a	25.5 a	1.98 a	12/1	3.14 b	0.09 a	2.26 a	0.55 a
0.0 (with earthworms)	7.73 c	25.1 a	2.19 a	11/1	3.45 a	0.08 a	2.15 a	0.56 a
0.0 (Without earthworms)	7.78 b	23.2 a	1.78 a	13/1	2.23 c	0.05 b	1.71a	0.41 b
CV %	0.63	8.30	9.06		3.23	0.59	6.80	3.0

Means followed by the same letter, in column, do not differ by Scott Knott test at 5% probability. * x10¹¹ CFU kg⁻¹ doses

Soares (2004) conducted the chemical featuring of a commercial vermicompost and recorded phosphorus values ranging from 1.0% to 1.2% in the analyzed samples; these values were lower than those found in the current study (Table 1). Lazcano et al. (2008) also found increased phosphorus values in the presence of earthworms in a vermicomposting study. Potassium rates varied between 0.08 and 0.09 in trichoderma and earthworm treatments; these treatments showed significant potassium increase in comparison to the control treatment without earthworm and without trichoderma (0.05%). The magnesium rate (0.41) was lower in the control treatment without earthworms and trichoderma than in the other

treatments, which did not show variation among them (Table 1). Results recorded by Lamim (1996) for vermicomposting using cattle manure showed lower K (0.02%) and higher Mg (0.44%) than the control treatment without trichoderma and earthworm, although both results were lower than those recorded in the other treatments.

The waste decomposition and transformation process in most treatments resulted in C/N ratio of approximately 12/1 (Table 1), which is below the 18/1 ratio that, according to Kiehl (1985), indicates compound maturity. Results similar to those found in the current study were recorded by Soares et al. (2004), who found values between 9.02 and 13.74. Antonioli et al. (2002)

studied several vermicomposts exclusively derived from cattle manure produced in different counties in Rio Grande do Sul State and found C/N variations between 7 and 33.

CONCLUSIONS

According to the survival curve of *E. andrei* individuals, the results recorded in the lethality test showed decreased number of earthworms only when ICB doses equal to or higher than 4.0×10^{11} CFU kg^{-1} were used.

High doses of the biological product ICB nutrisolo *Trichoderma* - up to 1.0×10^{11} CFU kg^{-1} - did not change the number of adult earthworms and the number of *E. andrei* cocoons in the vermicomposting using cattle manure; however, the

multiplication index was lower in all ICB treatments. ICB doses above 2.0×10^{11} CFU kg^{-1} decreased the total dry weight.

There was increased P, K and Mg rates and little pH variation in the treatments with earthworms.

The C/N ratio in all the treatments indicated organic compound maturity within the acceptable levels.

ACKNOWLEDGMENTS

The authors thank the company ICB BIOAGRITEC LTDA and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES - Brazil).

RESUMO: A eficácia da vermicompostagem e de bioprodutos à base do fungo antagonista e promotor de crescimento vegetal *Trichoderma* (*Trichoderma* spp) é bem conhecida e estudada na agricultura orgânica. Entretanto, para uma melhor eficiência metodológica, são necessários estudos que possam avaliar a interferência de altas doses desses bioprodutos na biologia e desenvolvimento das minhocas. Baseado nesse contexto, o objetivo deste trabalho foi testar altas doses do produto comercial biológico ICB Nutrisolo *Trichoderma* (ICB), avaliando-se a multiplicação e desenvolvimento de *Eisenia andrei*, bem como alterações nas características químicas do substrato produzido no processo de vermicompostagem, a partir do resíduo orgânico esterco bovino. O esterco bovino *in natura* foi autoclavado a 121°C, por duas vezes, em um intervalo de 24 h. A unidade experimental constituiu-se de 6 kg de substrato condicionados em caixa multiuso de polipropileno, com dimensões 20 x 40 x 50 cm, contendo 48 minhocas adultas e cliteladas da espécie *E. andrei*. Como agente biológico, utilizou-se o produto comercial ICB na forma de fluído, composto por oito cepas das espécies *T. koningiopsis*, *T. asperellum* e *T. harzianum*, com as seguintes doses nos tratamentos a seguir: T1 (0.5); T2 (1.0); T3 (2.0); T4 (4.0); T5 (8.0); e T6 (0.0), sendo todas as concentrações em 10^{11} UFC kg^{-1} do produto em vermicomposto, e, para a avaliação das características químicas do vermicomposto em altas doses do produto ICB, foi utilizado também o T7 (somente substrato). O delineamento foi inteiramente casualizado com quatro repetições por tratamento. A temperatura foi mantida a 28°C e a umidade entre 60 e 70%. Após 60 dias do início da instalação, fez-se a contagem do número de minhocas adultas, jovens e casulos; posteriormente, avaliou-se o seu peso seco total. Os resultados observados no teste de letalidade mostram que, somente a partir de 4.0×10^{11} UFC kg^{-1} de ICB, há decréscimo do número de minhocas. Doses altas até 1.0×10^{11} UFC kg^{-1} do produto não alteram o número de minhocas adultas e de casulos de *E. andrei* em vermicompostagem com esterco bovino, entretanto, o índice de multiplicação foi inferior em todos os tratamentos com o produto. Doses acima de 2.0×10^{11} UFC kg^{-1} diminuíram o peso seco total. A relação C/N em todos os tratamentos indica maturidade dentro de resultados aceitáveis para compostos orgânicos.

PALAVRAS-CHAVE: *Trichoderma* spp.. Produto biológico. Minhoca. Vermicompostagem.

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