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RESEARCH ARTICLE

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ORGANIC FERTILIZER STRATEGIES FOR THE PRODUCTION OF STRAWBERRIES AN AGROECOLOGICAL SYSTEM

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ABSTRACT

Introduction: among the organic strategies of agricultural production is composting. The aim of this study was to develop an organic fertilizer from horse manure and test it in the production of strawberries. Strawberry seedlings were planted, containing horse manure, birds, chemical fertilizers and only ravine soil. Soil samples were used to perform a chronic toxicity test. After harvesting, physical and antioxidant analyzes were performed on the fruits. Results: in the analysis of soil toxicity, a greater reproduction of colemolans was observed in the soil containing bird compost. As for physical-chemical analysis, strawberries produced with chemical and equine fertilizers had a lower moisture content. Strawberries produced with horse compost had a higher carbohydrate content. The horse compound helped to increase the presence of total phenolic compounds and the antioxidant activity of the produced strawberries. Conclusion: thus, the use of equine composting can be considered a sustainable option for the production of strawberries.

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INTRODUCTION

Brazil is among the largest pesticide consumers in the world, due its great dependence of agriculture production, mainly with regard to strawberry production, since this fruit is among the most contaminated by this substance. In addition, in this scenario, the southern region of the country appears as the second place among the regions that consume agrochemicals. The state of Paraná reaches the third place among the Brazilian states that use these substances (INPE, 2016; PIGNATI et al., 2017; LOPES & ALBUQUERQUE, 2018). Moreover, a worrying fact about the use of agricultural pesticides is that most have the ability to act as endocrine disruptors in humans and animals. This fact becomes a major public health problem (CASSAL et al., 2014; GRIMALDI et al, 2015). Thus, more sustainable agriculture alternatives are sought, such as the organic production system, that it aims to encourage the use of organic compounds to improve soil quality, besides it assists in the natural control of pests (FALCÃO et al., 2013). A significative part of Brazilian agricultural production is from family agriculture, where the family is owner of its land and means of production (NETO & BASSO, 2011). Also, it is known that organic production generates many benefits for family agriculture, as the productive diversification

in the property; the generation of more jobs, because it requires more manpower; lower production cost, due it does not use pesticides. Besides, longer shelf life in the post-harvest period (BARBOSA & SOUSA, 2012). The composting system represents an efficient solution for recycling organic materials, in view that it incorporates easily into the soil and it assists in the agricultural production process, due the quantities of chemical fertilizers used are reduced (GOMES et al., 2012). Also, according to data from the International Federation of Organic Agriculture Movements, organic agriculture is based on a pillar of various principles, including health, ecology, equity and environment. Furthermore, this type of production is considered safer and more sustainable by the producers, since is based on direct communication among soil, plants, animals, people, ecosystem and environment. Moreover, it is free of agrochemicals and genetically modified organisms (IFOAM, 1998). However, some factors are still limiting the adherence of organic production, such as the reduced level of farmers education and restrict access to technical assistance and participation in social organizations (BARBOSA & SOUSA, 2012). Currently, according to research conducted by the Brazilian Institute of Geography and Statistics (IBGE) in 2013 Brazil presented a population around 5.312.076 horses heads, that directly and indirectly generated around 3 million jobs (MAPA, 2016). Also, each

equine weighing on average 385kg produces daily about 16.3kg of manure that can be transformed in agroecological compounds for organic production (TRANI et al., 2008). Although, the data show that only 22.5% of Brazilian municipalities have organic production (MAPA, 2016). According to Embrapa (2006), the cattle and equines are the richest in fibers and therefore support the development of microorganisms that antagonize the action of fungi harmful to the soil. On the other hand, the dung cannot be used fresh, once, besides containing undesirable microorganisms for human health, it can burn the plant. Thus, the manure must first pass through a fermentative process, such as the composting process. In this way, composting is an excellent agroecological strategy, due it assists in improving soil quality and maintaining fertility, humidity and temperature at adequate levels. Aerobic composting with manure is an excellent tactic because it is able to promote the preservation of the environment and the health of producers through its production process. During the fermentation phase occurs an increase in the temperature and release of odorless gases and vapors present in the compounds, resulting in agroecological fertilizers (NUNES, 2009). According to Darolt (2008) some plants are more likely to be contaminated with pesticides, due to the lack of respect to the grace period between the application of the substance and the sale of the product. An example is the strawberry (*Fragaria X Ananassa* Duch), a fruit much appreciated by the Brazilian population and produced on a large scale in Brazil (MUSA et al., 2015; FALCÃO et al., 2013).

In relation to data from the Secretariat of Agriculture and Supply of Paraná in 2015, the strawberry was cultivated in 723 hectares, where it was produced around 22600 tones, contributing with 11.7% in the gross value of the production of Paranaense fruticulture. In addition, strawberry presents two periods that are regulated by photoperiod, long days and high temperatures, where the vegetative phase occurs, with stimulation of the plant for the production of the seedlings and; still, the short days and milder temperatures to promote flowers and fruit production (FILHO & TIVELLI, 2017). This fruit is produced in several regions of the world and is available all year, in view of the existence of a wide diversity of systems for its production (FILHO & TIVELLI, 2017). Also, the demand for new technologies involved in the fruit production sector has growing, in search of improvements in productivity and quality of products, as well as in the health of producers (SEDIYAMA et al., 2014). Thus, an alternative for the improvement of both production and quality of organic strawberries would be the use of composting. In addition to being of great economic importance, the strawberry presents a very relevant characteristic for organic production, the fact that it is a perennial fruit, that is, if not attacked by diseases and pests it can last for years (FILHO & TIVELLI, 2017). Also, this fruit has very important nutritional characteristics, such as the presence of vitamin C, that presents antioxidant action, that is, it is able to scavenge free radicals produced in the human body, substances responsible for a phenomenon denominated oxidative stress (EO) (MUSA et al., 2015; MARRONI, 2008; COUTO & CANNIATTI-BRAZACA, 2010). However, researches show whereas most marketed strawberries present levels of agricultural pesticides above of the tolerated limits (GORENSTEIN, 2000; AMARAL & ALTOÉ, 2005). And research has also shown that these agrochemicals in the organism have the ability to increase EO (MARTINI et al., 2016). Therefore, the consumption of this fruit becomes infeasible to health if it presents contamination with agrochemicals. Furthermore, a solution to this problem would be the implantation of organic production, using agroecological technologies, minimizing the harm to health and the environment caused by pesticides (MUSA et al., 2015). Therefore, organic production using horse fertilization can generate benefits for fruit production, such as strawberry, in view that its power antagonizes fungi that cause diseases in this crop.

MATERIAL AND METHODS

Methods Used for Agroecological Production

Soil used for planting: For the planting of both groups of strawberries, the soil of the ravine was used, acquired in the

experimental area of the Federal University of 'Fronteira Sul' – Campus Realeza. In addition, in all groups were used limestone, in order to correct the pH of the land.

Aerobic composting for organic fertilizer production: Aerobic composting containing equine manure was assembled in an open environment with the presence of oxygen. For the production of the compound, about 20% of carbon sources (straw and dry leaves) and 80% of a nitrogen source (equine manure) was added in alternative layers. After fermentation occurred the detachment of odorless gases and water vapors, resulting in an organic fertilizer that was used to cultivate strawberries. The temperature, humidity and aeration of the composting leira were periodically verified according to the Protocol of Nunes et al. (2009), as well as all the procedures described above.

Strawberry cultivar: The cultivar 'San Andréas' was choose for this study, because it is possible its in natura consumption, due the good fruit quality, both in terms of flavor, size, firmness and bright red color. In addition, the plant has a medium size, that facilitates the harvesting and its leaves are of larger sizes and thickness. These factors contribute to produce three to four tillers in the first year, it providing better fruit size and quality (24). The seedlings were commercially acquired from Floriculture Silvestrin localized in the municipality of Coronel Freitas - SC - Brazil.

Planting Techniques: 200 plastic pots were used, with capacity around 8 kg, where 200 strawberry seedlings were planted, according to the experimental procedure described above. A seedling was planted in each vase to possibility sufficient space for its adequate growth and development. In addition, the selected seedlings presented the following characteristics: 2 to 3 healthy leaves (without fungi or other types of diseases), absence of any chemical input and those with the Crown (part where the root is with stem) with more than 4 mm. Furthermore, all the old or dead leaves were removed, besides it was performed the thinning of the roots, when its length found above 10 cm. At the time of planting the seedling, the root was well distributed in order to be uniformly dispersed, avoiding to curl or bend. Moreover, the depth was observed, because the seedlings could not be too buried or far above the ground (EMBRAPA, 2013).

Chronic Toxicity Assay with *Folsomia Candida*: All soils used for planting were subjected to analysis to ecotoxicological, using Collembolans *Folsomia candida*. The organisms were reared in the laboratory according to ISO (2014), under controlled conditions of temperature (20 ± 2 °C) and luminosity (12-h photoperiod). The effects of different treatments on *F. candida* reproduction were assessed through the ISO 11267 (ISO, 2014) recommendations. The test was carried out in cylindrical glass containers (7.5 cm diameter and 6.0 cm height) where 30 g of wet soil (witness) were added. Ten collembolans aged between 10 and 12 days, taken from synchronized cultures, were inserted into each container. Four replicates were prepared for each treatment. Organisms were fed with five milligrams of dried granulated yeast at the beginning and on 14th day of the assay. Containers were hermetically sealed and twice a week, they were opened for air renewal and soil moisture adjustment based on weight loss, in order to maintain the soil moisture close to 60% of water holding capacity of each soil. After 28 days of the start of the assay, the content of each replicate was carefully transferred to plastic containers and was immersed in water and black ink, in order to promote the fluctuation and facilitate visualization of the *F. candida* surviving juveniles. Each replicate containing the floating individuals were photographed in high resolution and the photos were analyzed on ImageJ[®] to account for the number of juveniles generated during the assay.

Techniques After Planting

Experimental procedure: All the phases of this study were performed in Federal University of 'Fronteira Sul' - Realeza, Paraná - Brazil. The development of organic fertilizer was performed in an experimental area. The strawberries planting, cultivation and harvest were conducted in a greenhouse. The pots were placed on metal

benches suitable for experimental studies, with a distance of approximately 1 meter from the ground and 20 centimeters of distance between the pots to create a uniform space among the seedlings. The physicochemical and antioxidant analyses of the strawberries were performed in the biochemistry and bromatology laboratories at the *Campus Realeza*, while the chronic toxicity assay with *Folsomia Candida* was performed at the *Campus Chapecó*.

Fertilization: For each experimental group, different fertilizations were used, as described below:

- Witness group: 8kg of soil and 20g of limestone;
- Chemical group: 8kg of soil, 20g of limestone and 10g of chemical fertilizer NPK 08-20-20, acquired at local commerce;
- Compound horse group: 6kg of soil, 20g of limestone and 2kg of organic fertilizer produced from equine, as previously described;
- Compound bird group: 6kg of soil, 20g of limestone and 2kg of poultry fertilizer, purchased at local commerce.

Irrigation: All the groups were constantly irrigated with drinking water to allow adequate nutrient absorption, growth and fruit production. The plants were irrigated on alternating days and when warmer days, daily, always in the morning. The water used was of good quality and without contaminants (EMBRAPA, 2013), acquired through the municipal supply network, treated by 'SANEPAR' (Sanitation Company of Paraná).

Pests and diseases monitoring: All groups were monitored daily in relation to the main pests and diseases that can attack the fruit, as described by Embrapa (2013), in view to avoid major infestations. Diseases monitored: plant wilted, caused by soil fungi (*Phytophthora* sp., *Fusarium* sp., *Pythium* sp. and *Verticillium* sp.); the leaf spots, such as the mildew (*Oidium* sp.); the stains occasioned by mycosferella (*Mycosphaerellafragariae*) and; the fruit rot, mainly caused by the gray mold (*Botrytis cinerea*). Pests monitored: aphgons; Lobiopa or strawberry borer and; caterpillar-thread.

Pests and diseases control: To control the pests and diseases of all groups was initially performed the elimination of the diseased parts of the plant. Subsequently, Bordalese syrup and neem oil diluted to 1% were applied every 15 days, used as fungicides, acaricides and fertilizer inputs allowed in organic production.

Harvesting Techniques: The strawberries of the four groups were harvested as described by Embrapa (24), about 60 to 80 days after planting and/or when the fruit was ripe, that is, with more than 75% of red color. After harvest the strawberries of each group were destined to the laboratory of biochemistry and bromatology of the Federal University of 'Fronteira Sul' Realeza - PR, where the fruits were crushed and stored in freezer (-18°C) until the moment of the analyses. The antioxidant and physicochemical analyses were performed in triplicate in all groups.

Physicochemical Analysis: To determine moisture, the samples were dehydrated until constant weight was obtained under a certain temperature. For this purpose, a greenhouse regulated at a temperature of 105 °C, porcelain crucible, dehydrator and analytical scale. For the determination of ash, the organic matter present in strawberries was burned at a temperature of 530 °C in a mufla oven. To determine the lipid fraction, the method described by IUPAC (1979) was followed, using the Soxhlet extractor to determine the lipid fraction of strawberries. For this, ether, Soxhlet extractor, Soxhlet cartridges, 105 °C greenhouse and analytical scale were used. And for the determination of proteins, the method described in AOAC (1995) was followed using the Kjeldahl digester. Nitrogen-free concentrated sulfuric acid, mixed catalyst (composed of 100g anhydrous sodium sulfate and 10g pentahydrate copper sulfate), hydrochloric acid solution 0.1 N, mixed indicator solution (composed of red of methyl 0.50% + bromocresol green 0.75% in ethyl alcohol), sodium hydroxide solution (50%) and 4% boric acid solution.

Finally, for carbohydrate determination, the difference or Nifext method was used, since the sum of all the components mentioned above in the sample is equivalent to 100%. Thus, the value of the carbohydrate fraction was represented by the following formula: Nifext = 100 - components.

Antioxidant Analyses: Antioxidant analyses were not performed in the group of strawberries produced with bird fertilising, since it presented a low production.

Fruit extracts: This procedure was adapted from Larrauri et al. (1997). 1g of the fruit sample of each group was weighed and introduced into a 100mL beaker; after 40 ml of 50% methanol was added, homogenized and waited for 60 minutes at room temperature. The material was centrifuged for 15 minutes. From the residue of the first extraction, 40 ml of 70% acetone was added to the supernatant, homogenized and allowed to stand for 60 minutes at room temperature. It was centrifuged again for 15 minutes. The final supernatant was transferred to a volumetric flask and completed to 100 mL with distilled water.

Determination of phenolic compounds: This technique was performed in both groups as described by Beal (2006) and Budini et al. (1980), with some adaptations. The compounds were dosed using Folin-Ciocalteu reagent and measured at 740nm (for total phenols) and 427nm (for total flavonoids). The phenolic compounds analyzed were phenols and flavonoids, the main ones found in strawberries.

Determination of antioxidant activity by the ABTS method: The antioxidant capacity was determined by the reduction of the free radical ABTS (2,2'-azinobis (3-ethylbenzotiazoline-6-sulfonic acid) through the antioxidants present in the samples, according to the method proposed by Thaipong et al. (2006) adapted. Aliquots of 30 mL of sample were added in 3 mL of ABTS and measured in the spectrophotometer at 734nm. This analysis was performed in all experiment groups except the bird group, due to the low strawberry yield in this group.

Data Analysis: To perform the statistical analysis of the data, a database was created to subsequently perform the statistical analysis in the software GraphPad Prism® 6 (Prism 6.03, Software GraphPad, San Diego, CA, USA). Initially, descriptive statistics were performed and the results were expressed in mean and standard deviation. After performing One-Way ANOVA, since the data presented normal distribution and later, to verify the difference between the control group and the other groups, Tukey test was applied. A p<0.05 value was adopted for statistical difference.

RESULTS AND DISCUSSION

The fertilizer produced from equine waste proved effective for the production of strawberries, in addition, it presented similar characteristics, such as texture and color to organic fertilizers already commonly produced. A similar study conducted by Silva et al. (2018) demonstrated that the composting process generated more stable compounds in relation to temperature, besides it was used materials similar to this study, as leaves and dried straws, in addition to an organic compound. Moreover, it can be observed that compounds produced from animal waste showed promising results with regard to food production, besides to be an organic strategy for agricultural properties. According to soil and fertilizer analysis in relation to environmental aspects (Figure 1), it was observed that in the group where was used composed bird, there was a significant improvement in collembolans reproduction. Results similar to our study were observed by Lock & Janssen (2002), which found that the number of juveniles reproduction was increased after the use of a material as substrate for the soil. Thus, we can verify that when the substrate is rich, the reproduction of Collembolans increases. Both fertilization methods (bird and horse compostes) are considered rich substrates for food production, however, it is believed that the result found in ecotoxicology soil analysis may be explained by the composition of fertilizers, where, according to Raji et al., (1996) the average

chemical composition of fresh bovine manure is: 5 g/kg of nitrogen; 2.6 g/kg of phosphorus; 6 g/kg of potassium; 2 g/kg of calcium; 1 g/kg of magnesium; 1 g/kg of sulfur; 33 mg/kg of zinc; 6 mg/kg of copper and 2 mg/kg of nickel. And, since the average manure of fresh birds is: 14 g/kg of nitrogen; 8 g/kg of phosphorus; 7 g/kg of potassium; 23 g/kg of calcium; 5 g/kg of magnesium; 2 g/kg of sulfur; 138 mg/kg of zinc; 14 mg/kg of copper and 2 mg/kg. Furthermore, mineralization in soil through nutrients, mainly nitrogen and phosphorus, depends on the carbon/nitrogen (C/N) ratio of the organic material used, where according to Trani&Trani (2011) the C/N of bird manure is greater when compared to that of horse. Therefore, it can be observed that the bird compound has a higher nutrient content than the horse compound, explaining the results found.

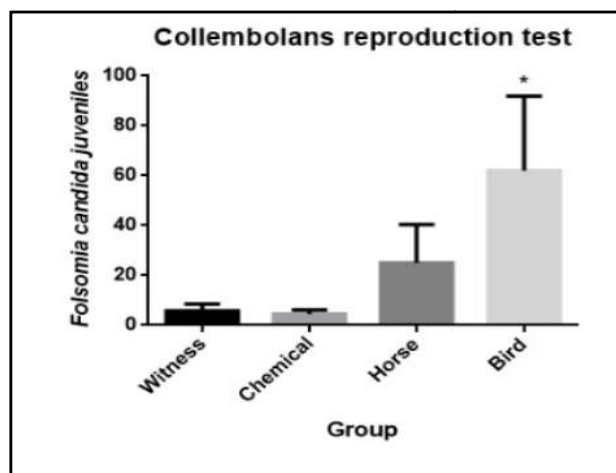


Figure 1. Collembolans Reproduction Test in the different soils used for the planting of strawberries

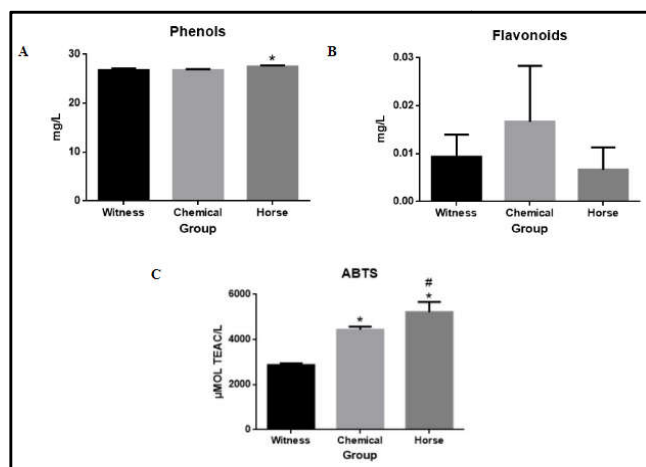


Figure 2. Antioxidant analyses performed in triplicate in strawberries planted with different treatments. Where A and B represent the total phenolic compounds and C represents antioxidant analysis by the ABTS method

As described in Table 1, it was found that the chemical and horse groups presented smaller moisture content in relation to the group of witnesses. According to the Brazilian Food Composition Table (2013), fresh strawberry has an average of 92% moisture and for Food Composition Table (2011), were observed an average of 91.6% of humidity. Besides similar results were also found in studies, such as Menezes et al. (2010), Giampieri et al. (2012) and Hossain et al. (2015), with values between 90.58% and 92.17% to humidity. It was observed that in our study the moisture content was considered lower, fact that may be explained by the cultivar used, type of planting, time of exposure to the sun, mode of irrigation, considering, that several factors can interfere in the analyses physicochemical of strawberries produced, especially with regard to the moisture. In addition, it was possible to observe that there was a higher quantity of carbohydrates in strawberries produced with horse manure and, similar results were

also observed in the data of the chemical composition table of foods to conventionally produced strawberry (2006). This can be explained by the cultivar used in the study, that presents a sweeter taste and is considered suitable for fresh consumption.

Table 1. Physicochemical analyses performed in triplicate with strawberries produced with different fertilizer treatments

	Witness	Chemical	Horse	Bird
Ashes	0,51 ± 0,26	0,72 ± 0,15	0,65 ± 0,47	0,57 ± 0,0
Moisture	86,83 ± 0,52	84,82 ± 0,72 *	84,09 ± 0,97 *	87,64 ± 0,59
Carbohydrates	12,26 ± 0,64	14,68 ± 0,69	15,19 ± 0,95 *	11,79 ± 0,59
Proteins	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Lipids	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Moreover, another factor that may have interfered with the increase in carbohydrate value may be due to the fact that it no identified proteins and lipids in the samples of the strawberries here studied. Hossain et al. (2016) verified in its study the presence of small values of proteins and lipids in strawberry samples, about 0.57 to 1.17g/100g of sample and 0.33 to 0.48 g/100g of sample, respectively, which corroborates the data found in this study. Additionally, according to Schwieterman et al. (2014), the purchase intention is very interconnected with factors such as color, flavor, aroma, texture, and; the taste in general is influenced by the intensity of the fruit sweetness. In this sense, the more carbohydrate (fructose) the fruit possess, greater the product purchase intention. Thus, to keep and improve the strawberry flavor, an extremely delicate fruit, is important and relevant to the food science and technology. Given the above, we highlight the relevance of the use of organic compounds for strawberry production, especially the horse compound, since it presented a statistically higher carbohydrate content than the control and chemical group. In relation to the amount of ash present in groups produced with different fertilizers, no significant difference was observed. Besides, the content of lipid and protein in the samples, there were no detectable. This result was also observed in the Food Composition Table (2011) and USDA (2006), where the values of ash, lipids and proteins are low when compared to carbohydrate values. In relation to the total phenolic compounds, it was observed that there was a significant difference in the group of strawberries planted with the horse compound. However, in the total flavonoids content there was no significant difference among the treatment groups. According to the literature, phenolic compounds are widely discussed today, given that they are present in a considerably large amount in certain products, however, their bioavailability and absorption are not yet entirely confirmed.

Therefore, new forms of production have been studied, in view to assist in this aspect (ARIZA et al., 2018). When comparing antioxidant activity, it was observed that both strawberries produced with horse fertilizers and chemist presented superior antioxidant activity in relation to witness group, according to ABTS method. Besides, the strawberries produced with horse fertilizer demonstrated higher increment in antioxidant capacity when compared to chemical group. Duarte-Almeida et al. (2006) observed in their study that acerola extract presented the highest antioxidant activity, followed by extracts of blackberry, acai and strawberry, but the method used to verify antioxidant activity in this study was DPPH. Moreover, it should be considered that in this study conventional strawberries were used for the analysis, where, maybe, if there were the use of organic strawberries, such as those produced with horse manure, the fruits could rise in this ranking. In addition, studies that verify the antioxidant activity of strawberries by the ABTS method are still quite scarce. From this study, it can be concluded that horse manure is an excellent option for organic production, since it contributes to the production of strawberries with high levels of carbohydrates, in addition to greater antioxidant activity. Moreover, it can be considered that these findings could contribute to the increase strawberries in market value, both organic products in general and organic strawberries.

Competitive Interests: We declare that there is no conflict of interest in this scientific article, just as we agree with publication in Nutrition Journal;

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