horticultura brasileira	Research

BASTOS, FEA; RIBEIRO, SB; BORBA, AF; CAMPOS, LT; FINCATTO, D; BERTOLI, CD. 2022. Use of rabbit manure in lettuce culture: an alternative option. *Horticultura Brasileira* 40: 367-372. DOI: http://dx.doi.org/10.1590/s0102-0536-20220403

### Use of rabbit manure in lettuce culture: an alternative option

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#### ABSTRACT

#### **RESUMO**

Organic fertilization favors the physical and chemical attributes improvement of the soil and promotes the use of waste that would be discarded in the environment. This study aimed to identify the appropriate levels of rabbit manure compost (RMC) from rabbit farming in the production of substrates for lettuce. The experiment was implanted in plastic pots using 5 treatments: 0%, 25%, 50%, 75% and 100% of RMC mixed with commercial substrate, in a completely randomized design with 10 replications. The analyzed variables were number of leaves per plant, shoot green matter, root green matter, shoot dry matter and root dry matter. There was an increase in number of leaves with the increase of the RMC doses incorporated into the substrate, up to the dose of 59.46%. The shoot green matter had its best response on 61.42% of RMC. In both variables there was a decline afterwards. The root green matter started with an increase up to the dose of 36.14% of RMC, followed by a decline, and another increase, not reaching the same values obtained at the beginning of the curve. An increase was observed in the shoot dry matter as the amount of RMC increased, up to the limit of 45.09%, and then declined. The root green matter showed an increase until approximately 25% of RMC, stabilizing on 75% of the compost. In general, values close to 60% of RMC mixed with the substrate presented the best results. We can conclude that the RMC can be used to promote a greater number of leaves, which are the economically viable parts of lettuce culture.

Keywords: *Lactuca sativa*, composting, agro-industrial waste, organic horticulture.

# Uso de esterco de coelhos na cultura da alface: uma opção alternativa

A adubação orgânica favorece a melhoria de atributos físicos e químicos do solo e promove a utilização de dejetos que seriam descartados no meio ambiente. Este trabalho teve como objetivo identificar os níveis adequados do composto de esterco de coelhos (CEC) proveniente da cunicultura, na produção de substratos para a alface crespa. O experimento foi implantado em vasos plásticos utilizando 5 tratamentos: 0%, 25%, 50%, 75% e 100% de CEC misturado a substrato comercial, em delineamento inteiramente casualizado com 10 repetições. As variáveis analisadas foram número de folhas por planta, massa verde da parte aérea, massa verde da raiz, massa seca da parte aérea e massa seca da raiz. Para a variável número de folhas houve incremento com o aumento da dose de CEC incorporado ao substrato, até a dose de 59,46%. A massa verde de parte aérea teve sua melhor resposta na dose de 61,42%. Em ambas as variáveis houve declínio em seguida. Já a massa verde de raiz iniciou com incremento até a dose de 36,14% de CEC, seguido de declínio, e novamente aumento, não atingindo os mesmos valores obtidos no início da curva. Com relação à massa seca de parte aérea percebeu-se incremento das medidas conforme aumento da dose de CEC, até o limite de 45,09%, apresentando posterior declínio. Já a massa verde de raiz mostrou aumento até a dose de aproximadamente 25% de CEC, com estabilização a partir da dose 75% de composto. De modo geral, os valores próximos a 60% de CEC misturado ao substrato, apresentaram os melhores resultados, podendo concluir que o CEC pode ser utilizado para promover maior número de folhas, que são as partes economicamente viáveis da cultura da alface.

Palavras chave: *Lactuca sativa*, compostagem, resíduos agroindustriais, horticultura orgânica.

#### Received on May 9, 2022; accepted on September 2, 2022

Lettuce (*Lactuca sativa*) is among the most popular crops in Brazil (Vilela & Luengo, 2017), accounting for 40.9% of production within an economic chain with more than 174,000 establishments producing leafy vegetables, production of 908,186 tons of lettuce per year and 108,603 production units (IBGE, 2017).

The lettuce culture has great

importance in human food, standing out as a source of vitamins and minerals, being the most popular leafy vegetable consumed in the country (Ziech *et al.*, 2014). This leafy vegetable is exploited throughout the country and makes up an important portion of vegetables in the population diet, both for taste, low cost and nutritional quality, as a source of vitamins, minerals and fibers (Trentini & Hojo, 2019).

The cultivars, the production system and the handling in lettuce production vary within the region and property technological level, being able to adopt the conventional, organic or agroecological production systems, in protected crops (tunnels covered with meshes, screens, greenhouses) or those at field level (Barros & Cavalcante, 2021).

For leafy vegetables cultivation, the fertilization quality is fundamental, and the availability of organic fertilizers from animal manure can be considered a sustainability factor within the agricultural production system (Bonela et al., 2015). Such sustainability is based on the economic, social and environmental tripod, showing that rabbit manure production can generate income, maintain activities, consequently, the man in the field, and cycle cuniculture waste. Vegetable seedlings need a substrate of good physical, chemical and biological quality, determining the final quality of the crops (Antunes et al., 2019), conferring, for example, better conditions in the postharvest period (turgidity of the leaves and longer shelf period) due to the good nutrition of the plant. As lettuce needs a large amount of nutrients, planting the crop in soil with high levels of organic matter is ideal, raising productivity rates (Farias et al., 2017).

Many studies testing organic fertilization in lettuce culture have been published (Silva et al., 2010; Peixoto Filho et al., 2013; Goulart et al., 2018), however, research on the use of rabbit manure as a source of nutrients in organic crop fertilization in Brazil is rare. Rabbit manure can be used in the substrate for lettuce production, as it has favorable physical and chemical characteristics, such as presence of macro and micronutrients, lightness, aeration and organic matter (Pereira et al., 2020; Bassaco et al., 2019). The prepared substrate with rabbit manure can be an alternative to produce vegetables, presenting good performance, lowering production costs and reducing environmental impacts.

Rabbit manure has higher values of organic matter, carbon and nitrogen in relation to sheep, poultry and cattle manure, and higher value of organic nitrogen in relation to cow and ostrich manure (Moral *et al.*, 2005). Its average composition is 1.5 to 2.5% N, 1.4 to 1.8% P and 0.5 to 0.8% K (Machado & Ferreira, 2011). Rabbit manure, if already well tanned, can be used and commercialized as plant fertilizer. The tanned manure of rabbits can be intercropped to the cultivation of earthworms. Horticulturists, including florists, are very fond of rabbit manure. So, it is an alternative source of income for rabbit breeders (Machado, 2012).

The objective of this study was to evaluate the productive performance of lettuce in relation to various doses of manure compound of rabbits mixed with a commercial substrate.

#### **MATERIAL AND METHODS**

The experiment was implemented on March 10, 2020 on the premises of the Department of Olericulture of the Federal Institute of Santa Catarina, Campus Camboriú (IFC Camboriú) (48°1'33"S, 48°39'18"W, 6 m altitude). The climate of Camboriú is classified as humid subtropical; according to Köppen Geiger: Cfa. The annual average temperature is 20.4°C, with relative humidity ranging from 80 to 84% and annual rainfall of 1,768 mm.

The tested material consisted of vermicomposting mixture of rabbit manure and wood chips. This material consists of the waste generated by the rabbit production of the IFC Camboriú, where there is a concrete ditch, located below the rabbit cages, for manure collection, with 1.20 x 14.4 m dimensions. The depth varies from 20 to 50 cm, depending on the slope to flow the liquid waste (urine + slurry + any leftovers from the automatic drinkers) produced by the animals. The liquid is drained into a septic tank. We added red California earthworms (Lumbricus rubellus) as soon as there is enough organic matter for their permanency in fecal ditches. This material was periodically revolved (approximately once a month), for approximately a 12-month period. When stabilized, the ditches are emptied, cleaned and disinfected and the produced material is sieved and bagged. At the end of the process, the number of earthworms was approximately 1.5 thousand per square meter. We considered the compound stabilized at temperatures below 35°C inside the compost; when

there is a reduction of 1/3 from the original volume; when the mixture is homogeneous in relation to the degraded constituents, it is not possible to identify them, and presenting a musty earth smell, as described by Souza & Alcântara (2008). This resulting material is what we call the Rabbit Manure Compound (RMC) and was mixed to commercial substrate in due proportions for this research.

Five doses of RMC were tested in the substrate composition for growing lettuce seedlings. The applied treatments were: 0%, 25%, 50%, 75% and 100% of RMC produced in the Cuniculture Oriented Professional Practices Laboratory (OPPL) of IFC Camboriú, mixed with the commercial substrate (ash, pine bark, vermiculite and sawdust).

Samples from the RMC were sent for chemical analysis and micronutrient characterization to the Soil Analysis Laboratory of the Agricultural Research Company and Rural Extension of Santa Catarina (EPAGRI), located in Chapecó. The chemical analysis of the RMC showed the following values: pH= 5.8; Organic matter = 21.6%; Clay = 48%; Mg = 6.4 cmolc/dm<sup>3</sup>; P = 203.1 mg/dm<sup>3</sup>; K = 533 mg/dm<sup>3</sup> and Ca = 17 cmolc/ dm<sup>3</sup>. Micronutrient analysis detected the presence of Cu = 1.5 mg/dm<sup>3</sup>; Zn = 28 mg/dm<sup>3</sup>; Mn = 7.5 mg/dm<sup>3</sup>; Fe >5.0 mg/dm<sup>3</sup>.

The substrate used together with the RMC had in its composition: pine bark, ash, vermiculite, sawdust and biostabilized, with acidity corrective additives (0.50%), phosphate (0.50%) and NPK (0.50%). Its guarantees are electrical conductivity of 0, 50 +/ 0.30 mS/cm; pH 6 +/ 0.5; humidity 58%; WHC (water holding capacity) 90% and density 310 kg/m<sup>3</sup>.

Lettuce seeds, cv. Vera, were obtained from "Mandarin Mudas" company, located in Rio dos Cedros-SC, on March 10<sup>th</sup>, 2020. Seeds were sown in commercial substrate (Carolina type) in styrofoam trays with 288 cells. Lettuce seedlings were transplanted with 5 cm average size and 6 complete leaves, approximately 40 days after sowing.

Each treatment consisted of ten

replicates, totaling 50 pots, 1.2-liter capacity, randomly distributed on wooden pallets, under greenhouse conditions, covered by transparent plastic film, forming a completely randomized experimental design. Transplant took place on the same day for all vessels. Irrigation was by micro sprinklers, five times a day, giving a total volume close to 1 L of water per plant at the end of the cycle. Harvest was performed on April 26<sup>th</sup>, 47 days after transplantation, as well as the analysis of green variables.

Plants were washed for data collection to remove dirt, insects and remains of substrate attached to the roots. A cut was made close to the stem to separate shoots from roots. All leaves were detached from the shoot, separating them (whole leaves, regardless of the length, were counted for each repetition), and immediately weighed in a semi-analytical scale, using three decimals (model AD200 210g 0.001g with Chapel Mars); so, there was no moisture loss from leaves. The roots and stems were also weighed, and the shoot was composed by the sum of leaves and stem mass. After collecting the fresh data (number of leaves, shoot and root green matter), the material was dehydrated in an oven with controlled temperature at 60°C and weighed after matter stabilization for quantification of the shoot and root dry matter.

Obtained data were tested for normality by Shapiro Wilk statistical test, resulting samples with normal distribution for all tested variables. After that, data were submitted to polynomial regression analysis and tested for linear, quadratic, cubic and 4<sup>th</sup> order significance. The linear, quadratic and cubic analyses, when significant, had their inflection points calculated. For polynomial regression analysis, the highest significant degree was chosen, regardless of the minor degrees significance. For the statistical analysis we used the software R, Studio Cloud.

#### **RESULTS AND DISCUSSION**

Only the intercept of the regressions of shoot green matter and shoot dry matter were not significant. All other coefficients showed significance of 0.001 as well as the tests of comparisons between the different orders (degrees) of the regressions. The coefficient of determination of each regression was calculated and presented together with graphs and equations (Figures 1 to 5).

There was an increase for number of leaves and for the RMC dose incorporated into the substrate until reaching 59.46% of compost and, from there, a decline was observed in the number of leaves (Figure 1). In a study conducted with lettuce under different doses of organic fertilizer, the found results are in accordance with those of the present study, in which the number of leaves is proportional to the increase of organic fertilizer dose, up to the level of 100% of the recommended dose (Kölln et al., 2021). However, the authors emphasize that when raising the dose above the recommended for the culture, some negative factors may occur for the culture, such as predisposition to diseases, salinity stress, toxicity and soil acidification.

The shoot green matter also responded to 4<sup>th</sup> order significance, being the best response to the content of 61.42% of RMC, above that the shoot green matter tended to decline, showing that this would be the ideal amount of RMC (Figure 2). Pereira et al. (2020), in a lettuce culture study, affirm that the shoots green mass was positively influenced by the manure of rabbits processed by vermicomposting. This study showed an increase when compared to the substrate based on earthworm humus produced with bovine manure + 15%fine coal + 2% castor bean cake. corroborating with our findings. This is possibly due to the greater capacity of these substrates to replace the extracted or leached nutrients, which is indicated by the lower reduction



Figure 1. Crisp lettuce number of leaves as a function of rabbit manure compost doses in commercial substrate. Camboriú, IFC, 2021.



Figure 2. Crisp lettuce shoot green matter as a function of rabbit manure compost doses in commercial substrate. Camboriú, IFC, 2021.



Figure 3. Crisp lettuce root green matter as a function of rabbit manure compost doses in commercial substrate. Camboriú, IFC, 2021.



Figure 4. Crisp lettuce shoot dry matter as a function of rabbit manure compost doses in commercial substrate. Camboriú, IFC, 2021.

of electrical conductivity presented by these substrates throughout the seedling's development (Pereira *et al.*, 2020). The use of organic fertilization proved to be an interesting alternative in increasing the production of fresh mass in lettuce, agreeing with the results of several authors, in which an important factor evidenced is the characteristics improvement of the product to be consumed, producing plants with better quality than those grown exclusively with mineral fertilizers (Santos *et al.*, 1994; Silva *et al.*, 2010).

The cubic regression for the variable root green matter showed significance (Figure 3). The behavior of this variable begins with an increase in root green matter, up to the dose of 36.14% RMC, followed by a decline, which changes, and again increases, not reaching the same values obtained at the beginning of the curve, remaining the highest production of root green matter with the dose of 36.14% of compost in the substrate.

Saldanha & Ribeiro (2021), studying the use of avian litter in lettuce seedlings, obtained similar results in relation to the increase of root and shoot green matter, number of leaves as the poultry litter organic compost dosage increased, then successive decline, from which, the increase of compost began to impair the culture development. An explanation for this behavior can be the toxic effect that high doses of organic compost have on the lettuce culture; therefore, it is necessary to carry out other studies on the appropriate dosages of rabbit compost for each variety/ cultivar. Excessive nitrogen, present in the organic compound, causes a leaf limb burning, and loss of burned leaves, which reduces the commercial leaves, viable for trade and consumption

#### (Milhomens et al., 2015).

Regarding shoot dry matter, it increases as the RMC dose increases up to the limit of 45.09% in the substrate. followed by a decrease in the values for the highest doses. The regression analysis of this variable showed a significant 3<sup>rd</sup> order degree (Figure 4). The analysis of shoot and root dry matter is fundamental to infer the plant growth, as it is a very sensitive parameter to water fluctuations, since plants are most formed by water (Taiz & Zeiger, 2017) especially the lettuce crop. In an experiment also carried out with lettuce, Silva et al. (2010) found data that corroborate those of this study, stating that the decrease in production at higher doses of compost may have occurred due to the excess of mineralized nitrogen (N). High doses of N can cause phytotoxicity by releasing ammonium during the urea hydrolysis process, increasing the ammonium levels in the media (Milhomens et al., 2015). Lettuce responds well to N supply, a nutrient that requires special handling in terms of fertilization. Nitrogen deficiency delays plant growth (Silva et al., 2010) directly affecting chlorophyll and, consequently, plant photosynthesis, which may influence shoot dry matter values.

Regarding the behavior of the variable root dry matter, there was an increase up to a dose of approximately 25% of RMC (Figure 5). Visually observing the graphic representation of the regression of root dry matter, there is a tendency for stabilization from the dose of 75% of compost, which may be correlated to the best structure of the substrate, promoted by organic fertilization. This visual observation is justified by the difficulty of biological interpretation of  $4^{th}$  order regressions.

The oscillations found for root green matter and root dry matter may be from physicochemical characteristics of the RMC-based substrate, such as pH, conductivity or porosity. In a study carried out by Pereira *et al.* (2020), there were no significant differences between the substrates in relation to lettuce root fresh matter and their respective volumes, under greenhouse planting



Figure 5. Crisp lettuce root dry matter as a function of rabbit manure compost doses in commercial substrate. Camboriú, IFC, 2021.

conditions, using rabbit manure. Organic compost is known as an excellent soil conditioner due to its large amount of organic matter (Massukado, 2016), and this characteristic increases soil permeability, reduces erosion risks and helps to control moisture. These changes in soil structure are reflected in a better development of the plant root system (Veras et al., 2019). The same authors affirm, in their comparative study of chemical and organic fertilization, in lettuce, that the average of root production were statistically different, in which the best results were obtained for lettuce cultivated with organic compost. Organic compounds, when incorporated into the substrate, give it a greater capacity for formation and stability of soil aggregates, preventing, therefore, erosion processes. They also provide the necessary macro- and micronutrients for the culture and have potential to correct the soil/substrate toxicity and increase water infiltration and soil aeration (Donato et al., 2021).

Considering all analyzed variables, the treatment with 0% RMC was not very efficient in the production of curly lettuce, producing less when compared to its use in total dose of 100%. Neither did the 100% dosage prove to be ideal in any of the evaluated characteristics. In general, organic compounds provide an increase in soil organic matter and doses of phosphorus, calcium and potassium, as found in the chemical analysis of rabbit compost in this study, being very efficient in improving soil chemical properties, such as pH, sum of bases, cation exchange capacity and base saturation, reducing potential acidity (Oliveira *et al.*, 2014). The use of vermicompost in the substrates production allows obtaining quality lettuce, demonstrating that the use of this input can be considered a promising practice in the production of this vegetable in organic systems (Teodoro *et al.*, 2016). This result could be explained because the lettuce root system is expressive in the soil surface layer; therefore, it is the most fertile layer, favoring the nutrient absorption.

It is concluded that for number of leaves the ideal amount of rabbit manure compost is 59.47%, for shoot green matter is 61.42%, for root green matter is 36.14%, for shoot dry matter 45.09% and root dry matter 25%. It is recommended to use values close to 60% of rabbit manure compost mixed with the substrate to obtain the highest production.

#### ACKNOWLEDGMENTS

To "Mandarin Mudas", from Rio dos Cedros-SC, who kindly provided the lettuce seedlings for the experiment. To CNPq, through the PIBIC-EM grant and to IFC Camboriú for the structure and personnel provided.

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