Application of Cow Urine as a Liquid Biofertilizer in Carrot Production in an Agro-sustainable System

 Sinara Patrícia Mendes da Costa¹, Thaisa Aparecida Neres de Souza¹, Josiane Cantuária Figueiredo², Maria Josiane Martins¹, Débora Souza Mendes³, Flávia Soares Aguiar¹, Maristella Martineli¹, Isabelle Carolyne Cardoso¹, Renato Martins Alves¹, Polyana Danyelle dos Santos Silva¹,
Camila Maida de Albuquerque Maranhão¹, Amanda Maria Leal Pimenta¹, Guilherme Reis de Souza¹, Carlos Augusto Rodrigues Matrangolo¹, Fábio Cantuária Ribeiro¹ & Regina Cássia Ferreira Ribeiro¹

¹ State University of Montes Claros, Janaúba, Minas Gerais, Brazil

² Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil

³ North Fluminense State University, Campos dos Goytacazes, Rio de Janeiro, Brazil

Corresponding author: Maria Josiane Martins, State University of Montes Claros. Avenue Reinaldo Viana, 2630, Janaúba, Minas Gerais, Brazil. E-mail: josianemartins102012@hotmail.com

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Abstract

Carrot (*Daucus carota*) is one of the most important vegetables in the world. Cow urine is a fertilizer available in rural areas and can be used in agriculture. However, there are no indications of the best dose to be used in carrots. The authors aimed with this work to evaluate different concentrations of cow urine in the cultivation of 'Brasília' carrots. The treatments consisted of five doses of cow urine (0%, 0.5%, 1.0%, 1.5% and 2.0%) applied during the culture cycle. After 90 days, the agronomic characteristics (weight, length, diameter, productivity, luminosity, chromaticity, and Hue angle) were evaluated. The application of cow urine increased the weight, diameter, and length of 'Brasilia' carrots.

Keywords: Daucus carota, cow urine concentrations, productivity

1. Introduction

Carrot (*Daucus carota.*) is a vegetable of great economic importance. In addition, it is among the ten most produced in Brazil (Miranda et al., 2017). The application of fertilizers in management activities has become an important agricultural practice to improve soil fertility (Afrin et al., 2019), but in conventional carrot production systems, many inorganic fertilizers are used misused (Colombari et al., 2018). This increases the amount of agrochemicals applied to the soil (Chen et al., 2018). In addition to increasing costs for the farmer (Li et al., 2019).

The agro-sustainable cultivation of food is an alternative to these practices. Because, in addition to meeting food needs, it also improves the quality of the environment. It optimizes the use of natural resources (Imadi et al., 2016), promotes an increase in organic matter in the soil (Leon et al., 2018), and increases the microbiota responsible for several biological processes (Testani et al., 2020). In agro-sustainable production systems, residues serve as a source of nutrients (Diacono et al., 2019).

Among these residues is cow urine (Pradhan et al., 2018). Bovine excrement, cheap and easily available on our planet (Gupta et al., 2016). Cow urine contains 95% water, 2.5% urea, and 2.5% mineral salts, hormones, and enzymes (Mandavgane & Kulkarni, 2020). Other authors also mention that urine contains minerals such as calcium, sulfur, iron, phosphorus, manganese, and potassium (Sadhukhan et al., 2018), among other beneficial substances such as carbonic acid, ammonia, amino acids, enzymes, and cytokinins (Choudhary et al., 2017).

Cow urine is a source of improve soil fertility, productivity, and the quality of vegetables such as carrots (Verma et al., 2018). The application of cow urine improves soil texture and structure (Nwite, 2015). The use of cow urine corrects micronutrient deficiency (Kumar et al., 2018; Favorito et al., 2019) and makes phytohormones available to plants (Sharma et al., 2017; Ghosh et al., 2018). Therefore, cow urine is an effective tool to correct nutritional deficiencies (Kumar et al., 2017).

The creation of cattle in the north of Minas Gerais reaches 2.6 million heads (SEAPA, 2019) distributed among large and small producers. In other words, cow urine is an available resource and allows for the integration between livestock and food production (Napoles et al., 2018). Despite being an indicated practice for this type of production, there is still no research carried out in the semiarid region of Minas Gerais on the effect of cow urine on the yield of vegetables such as carrots. Therefore, the objective was to evaluate the effect of cow urine on the development of "Brasília" carrots in an agro-sustainable production system.

2. Materials and Methods

The researchers experimented in April, May and June 2016, in a greenhouse at the State University of Montes Claros, Janaúba campus, Minas Gerais. So that the experiment was free from the effect of rain and possible pests. In addition, the temperature was controlled (27 °C). The production methods, in addition to following the principles of agroecology, were applied by the determinations of Brazilian legislation (Law No. 10,831, of the Ministry of Agriculture, of 12/23/03, Brazil 2010).

We use wooden boxes measuring 0.60 m \times 0.35 m (0.210 m²), lined with black plastic canvas as containers for the production of carrots. In the wooden crates, we put type 2 sandy clay loam soil (0.200 m²). We sowed 50 seeds of carrot cultivar Brasilia per linear meter. After 30 days, we carry out thinning, leaving 24 plants per wooden box. We fertilize the plants with 200 g m⁻¹ of Bokashi organic compost (composition: 40% rice straw, 30% castor bean cake, 10% ripe banana peel, 10% efficient microorganisms grown in rich medium in the forest, 5% chicken manure, 2% bovine bone meal, 2% natural phosphate, 1% vegetable ash).

We collect cow urine during milking from cows raised in an agro-sustainable system in the municipality of Pai Pedro Minas Gerais, store it in a disinfected, sealed plastic container, and store it in a shelter for 3 days for fermentation to take place. The first application of cow urine occurred 34 days after planting. In total, there were nine applications at seven-day intervals. We use water to dilute cow urine according to each concentration.

The amount of water to be used was calculated on top of 2000 mL of final suspension. The calculation was performed as follows: 0% concentration (2000 mL of water + 0 mL of cow urine), 0.5% concentration (1990 mL of water + 10 mL of cow urine), 1% concentration (1980 mL of water + 20 mL of cow urine), concentration 1.5% (1970 mL of water + 30 mL of cow urine), concentration 2 % (1960 mL of water + 40 mL of cow urine). We use a spray bottle to apply cow urine. After 90 days of cultivation, we carry out the harvest. We removed 20 plants from the box, leaving the four borders.

We take the carrots to the Laboratory of Products of Vegetable Origin at the State University of Montes Claros and carry out the following analyses: diameter, length and weight of the root, luminosity, Hue angle, chromaticity, pH, soluble solids, titratable acidity, and SS/TA ratio-ripening ripening index-RI. We determined the stem diameter and root length with a digital caliper "Mitutoyo Absolute 150mm/6"-0.01mm/.0005" and root weight with a precision digital scale "Toledo Prix Lab As82/220r2".

We determined the root color by a "Minolta" colorimeter with reflectance reading of coordinates L* (luminosity), a* (red or green hue), and b* (yellow and blue hue), from the Hunterlab Universal Software system. Through the values of a* and b* we obtained the following values: Hue angle (Hue = tg⁻¹ b/a) and chromaticity (C = $(a^2 + b^2)$ 0.5). To read the pH, we used a sample of 10 grams of macerated carrots and homogenized it with 90 mL of distilled water. We took the reading in a pH meter "Digimed DM22". We determined the soluble solids by means of an "Instrutherm Model RT 90ATC" refractometer and its result was expressed in ° Brix. The titratable acidity was expressed in an equivalent gram of malic acid/100g of pulp.

We set up the experiment in randomized complete block design with 5 treatments (0%; 0.5%; 1%; 1.5% and 2% of cow urine) and 5 replicates. At concentration 0, we apply distilled water to the plants. Data were subjected to analysis of variance. Concentrations were subjected to regression analysis at 1% probability. Data were analyzed using Sisvar software (Ferreira, 2019).

3. Results

Tables 1, 2, and 3 describe the summary of the analysis of variance for the evaluated characteristics. The variables, soluble solids, titratable acidity, ratio, and pH were not influenced by the application of different concentrations of cow urine (p > 0.01). The variables length, weight, diameter, productivity, luminosity, chromaticity, and Hue angle were significant at 1% probability (p < 0.01).

Variation source	Medium Square			
	Length	Weight	Diameter	Productivity
Concentrations	55.14*	29121.89*	2.33*	0.832*
Block	0.50 ^{ns}	206.08 ns	0.10^{*}	0.005 ^{ns}
Error	0.59	302.51	0.03	0.008
Coefficient of variation (%)	9.57	23.48	8.37	23.43

Table 1. Summary of the analysis of variance for the variables, length, weight, diameter, and yield of "Brasília" carrots subjected to different concentrations of cow urine

Note. * Significant, ^{ns} not significant at 1% probability by the F test. Note.

Table 2. Summary of analysis of variance of variables, soluble solids, titratable acidity, ratio, and pH of "Brasília" carrots submitted to different concentrations of cow urine

Variation source	Medium Square			
	Soluble solids	Titratable acidity	Ratio	pН
Concentrations	1.26 ^{ns}	0.19 ^{ns}	32.61 ^{ns}	0.007 ^{ns}
Block	0.20 ^{ns}	0.02 ^{ns}	73.94 ^{ns}	0.004 ^{ns}
Error	10.66	0.02	67.95	0.006
Coefficient of variation (%)	11.32	5.70	9.51	1.24

Note. ^{ns} not significant at 1% probability by the F test.

Table 3. Summary of analysis of variance of the variables luminosity, chromaticity, and Hue angle of "Brasília" carrots submitted to different concentrations of cow urine

Variation source	Medium Square		
variation source	Luminosity	Chromaticity	Hue angle
Concentrations	46.06*	24.89^{*}	21.97*
Block	11.62 ^{ns}	3.42 ^{ns}	4.06 ^{ns}
Error	7.18	3.40	2.36
Coefficient of variation (%)	4.97	2.87	3.02

Note.^{*} Significant, ^{ns} not significant at 1% probability by the F test.

The length and weight of carrot roots showed linear behavior (Figures 1A and 1B). The same occurred with the productivity and diameter of carrots (Figures 2A and 2B). As the concentration of cow urine increased, the length, weight, productivity and diameter of carrots increased.

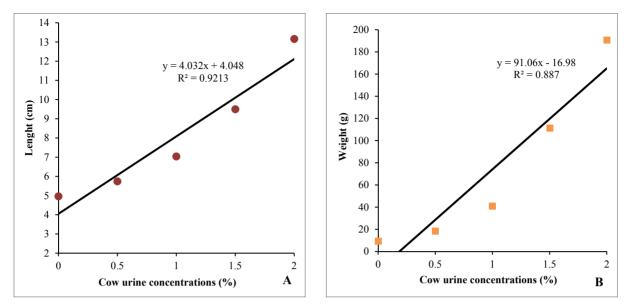


Figure 1. Length (A) and weight (B) of "Brasilia" carrots subjected to cow urine application

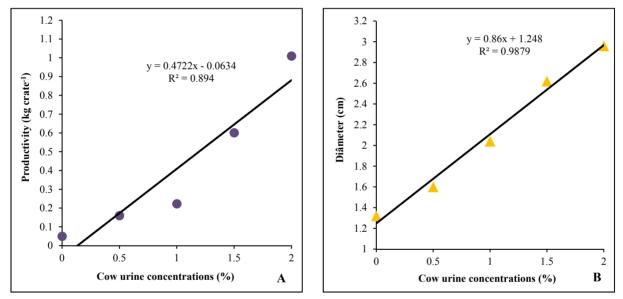


Figure 2. Productivity (A) and diameter (B) of "Brasilia" carrots subjected to cow urine application

The luminosity of carrots showed an inversely proportional linear behavior (Figure 3A). As the concentration of cow urine increased, the brightness of the carrots decreased. The chromaticity and Hue Angle increased as the concentrations of cow urine increased (Figures 3B and 3C).

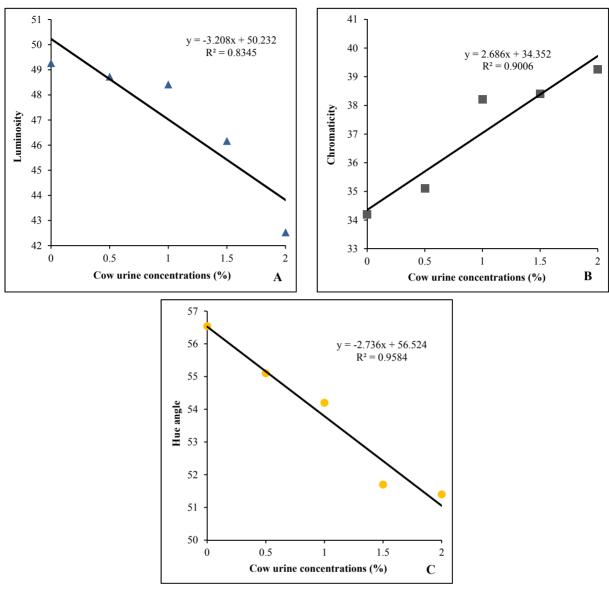


Figure 3. Luminosity (A), chromaticity (B), and hue angle (C) of "Brasília" carrots subjected to cow urine application

The increase in the length of carrots in relation to dose 0 was 15%, 41%, 91% and 165% for doses 0.5; 1; 1.5 and 2 respectively (Figure 4). For the weight of carrots, the increment was 91%, 328%, 1068% and 1902% (Figure 4). For diameter, the increments were 21%, 54%, 98%, and 124% (Figure 4). For productivity, it was 220%, 346%, 1102% and 1902% (Figure 4). In addition, for chromaticity it was around 2%, 11%, 12% and 14% (Figure 4).

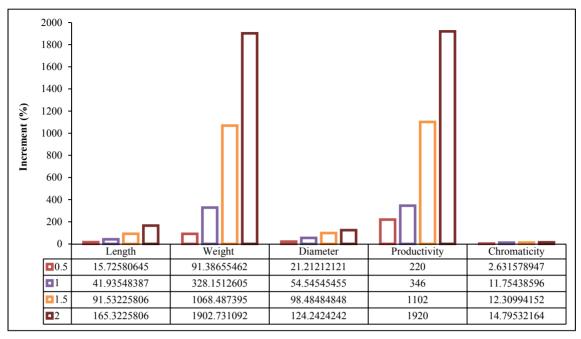


Figure 4. The increase in length, weight, diameter, yield and chromaticity of "Brasilia" carrots in relation to 0% concentration of cow urine

4. Discussion

The length of carrot roots ranged from 4.95 cm (0%) to 13.09 cm (2%), with an increase of 165%. Among the main effects of cow urine on plants is increased growth (Lovatto et al., 2011). This statement corroborates what was observed in this study.

Carrot weight was higher at 2% concentration. This result can be explained by the fact that cow's urine is composed of nutritional substances such as nitrogen, which has the function of promoting cell expansion and elongation (Jarvis et al., 1989). Similar results were verified by Araújo et al. (2014) in which applications of cow urine on peppers increased their size.

Oliveira et al. (2004) when working with peppers observed that productivity increased with increasing concentrations of cow urine. In a study with pumpkins, Costa et al. (2011) also observed an increase in production with increasing doses of cow urine. Oliveira (2007), when evaluating the influence of different concentrations of cow urine on the yield of 'Regina 2000' lettuce, observed an increase in production with an increase in the applied dose.

The largest diameter of the roots was 2.96 cm at the dose of 2% cow urine. These results can be explained by the fact that cow urine is an organic fertilizer rich in nutrients, including nitrogen. The results observed in the present work are in agreement with those found by Souza et al. (2011). The authors worked with onions under different concentrations of cow urine and found that the higher the concentration, the greater their diameter.

Regarding coloration, it is observed that carrots fertilized with concentrations of 0%, 0.5%, and 1 showed greater luminosity. These data are similar to those found by Boteiux et al. (2007) who, studying the color of Brazilian zucchini, observed that when there is a change in the color of the vegetable towards bright yellow to orange-yellow simultaneously with a reduction in L*, that is, the yellow becomes more opaque.

There was an increase in chromaticity with increasing doses of cow urine. It is observed that the roots cultivated with the 2% dose showed more intense color, which may be due to the greater accumulation of carotenoids. Grangeiro et al. (2012) found a less intense color (36.53) in the roots of "Brasília" carrots in a conventional system. This result can be explained by the fact that the carrots in our study were produced in an agroecological system and thus had higher levels of carotene.

Our results corroborate the results obtained by Neto et al. (2011) who found higher concentrations of carotenoids in carrots produced with organic compost. Oliveira et al. (2015) stated that the value of C* describes the

saturation or intensity of the color, in which products with higher values of C* are more vivid and, consequently, more attractive to purchase.

The Hue angle presented a linear behavior. Roots treated with doses 0%, 0.5%, and 1% had higher values, which indicates a more yellowish color, unlike the doses 1.5% and 2%, which had lower values, denoting an orange hue. Grangeiro et al. (2012) found roots with a higher Hue angle value, thus demonstrating yellower-colored carrots.

5. Conclusion

Cow urine increased the development of carrots cultivar Brasilia under the conditions evaluated.

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References

- Afrin, A., Islam, M. A., Hossain, M., & Hafiz, M. H. (2019). Growth and yield of carrot influenced by organic and inorganic fertilizers with irrigation interval. *Journal of Bangladesh Agricultural University*, 17(3), 338-343. https://doi.org/10.3329/jbau.v17i3.43207
- Araújo, D. L. (2014). Efeito de fertilizante à base de urina de vaca e substratos em plantas de pimentão. Revista Terceiro incluído, 4(2), 173-185. https://doi.org/10.5216/teri.v4i2.35270
- Boiteux, L. S., Nascimento, W. M., Fonseca, M. E. N., Lana, M. M., Reis, A., Mendonça, J. L., ... Reifchneider, F. J. B. (2007). 'Brasileirinha': cultivar de abóbora (*Cucurbita moschata*) de frutos bicolores com valor ornamental e aptidão para consumo verde. *Horticultura Brasileira*, 25(1), 103-106. https://doi.org/10.1590/ S0102-05362007000100020
- Brasil. (2010). Lei n. 12.305, de 2 de Agosto de 2010. *Institui a política nacional de resíduos sólidos* (Seção 1, pp. 30-31). Diário Oficial da União, Brasília.
- Chen, J., Lü, S., Zhang, Z., Zhao, X., Li, X., Ning, P., & Liu, M. (2018). Environmentally friendly fertilizers: A review of materials used and their effects on the environment. *Science of the Total Environment*, 613, 829-839. https://doi.org/10.1016/j.scitotenv.2017.09.186
- Choudhary, S., Kushwaha, M., Seema, S. P., Sodani, R., & Kumar, S. (2017). Cow urine: A boon for sustainable agriculture. *International Journal of Current Microbiology and Applied Sciences*, 6(2), 1824-1829. http://doi.org/10.20546/ijcmas.2017.602.205
- Colombari, L. F., & Lanna, N. B. L., Guimarães, L. R. P., & Cardoso, A. I. I. (2018). Production and quality of carrot in function of split application of nitrogen doses in top dressing. *Horticultura Brasileira*, 36(3), 306-312. https://doi.org/10.1590/S0102-053620180304.
- Costa, I. D. J. S., Souza, B. N., Fagundes, I. E., Oliveira, V. S., & Cunha, L. M. V. (2011). Avaliações de diferentes doses de urina bovina no desenvolvimento e rendimento agronômico da abóbora e milho consorciado com feijão. *Cadernos de Agroecologia*, 6(2).
- Diacono, M., Persiani, A., Testani, E., Montemurro, F., & Ciaccia, C. (2019). Recycling agricultural wastes and by-products in organic farming: biofertilizer production, yield performance and carbon footprint analysis. *Sustainability*, 11, 1-17. https://doi.org/10.3390/su11143824
- Favorito, A. C., Seide, E. P., Kohler, T. R., Carvalho, W. R., Pan, R., & Vasconcelos, E. S. (2019). Application of biofertilizers in cover of and its effects on organic maize. *Journal of Experimental Agriculture International*, 39(6), 1-7. https://doi.org/10.9734/JEAI/2019/v39i630349
- Ferreira, D. F. (2019). SISVAR: A computer analysis system to fixed effects split plot type designs. *Revista Brasileira de Biometria*, 37, 529-535. https://doi.org/10.28951/rbb.v37i4.450
- Ghosh, T., Biswas, M. K., & Guin, C. (2018). Efficacy of cow urine in wheat (*Triticum aestivum*) production as plant growth promoter and antifungal agent. *Journal of Pharmacognosy and Phytochemistry*, 7(3), 485-493.
- Grangeiro, L. C., Azevêdo, P. E., Nunes, G. H. S., Dantas, M. S. M., & Cruz, C. A. (2012). Desempenho e divergência genética de cenoura 'Brasília' em função da procedência das sementes. *Horticultura Brasileira*, 30(1), 137-142. https://doi.org/10.1590/S0102-05362012000100023
- Gupta1, K. K., Aneja, K. R., & Rana, D. (2016). Current status of cow dung as a bioresource for sustainable

development. Bioresources and Bioprocessing, 3(28), 1-11. https://doi.org/ 10.1186/s40643-016-0105-9

- Imadi, S. R., Shazadi, K., Gul, A., & Hakeem, K. R. (2016). *Sustainable crop production system*. Gewerbestrasse, Cham: Springer International Publishing AG. https://doi.org/10.1007/978-3-319-27455-3_6
- Jarvis, S. C. S., Hatch, D., & Roberts, D. H. (1989). The effects of grassland management in nitrogen losses from grazed award through ammonia volatilization; the relationship to excretal N returns from cattle. *Journal Agricultural Science*, 205-216. https://doi.org/10.1017/S0021859600085117
- Kumar, A., Baliyan, S., Kushwaha, A., Panwar, A., & Pundir, N. (2018). Review on the potentials of cow (Bos indicus) based bioenhancers in increasing crop yield and farmers income as well as the soil health and environmental sustainability. South Asian Journal of Research in Microbiology, 1(4), 1-16. https://doi.org/10.9734/SAJRM/2018/v1i4813
- Kumar, S., Datt, N., Sandal, S. K., & Sharma, S. K. (2017). Effect of cow urine and bio-fertilizers based fertigation schedule at varying levels of drip irrigation on yield, growth, quality parameters and economics of cucumber under protected condition. *International Journal of Current Microbiology and Applied Sciences*, 6(6), 1242-1249. https://doi.org/10.20546/ijcmas.2017.606.145
- Leon, N., Sun, Q., Utriainen, T., Nordberg, M., & Jones, R. (2018). Future states: design and science for sustainability. *Journal of Experimental Innovation*, 2(2), 13-24. https://doi.org/10.23726/cij.2018.765
- Li, Z., Zhang, R., Xia, S., Wang, L., Liu, C., Zhang, R., ... Liu, Y. (2019). Interactions between N, P and K fertilizers affect the environment and the yield and quality of satsumas. *Global Ecology and Conservation*, 19, 1-13. https://doi.org/10.1016/j.gecco.2019.e00663
- Mandavgane, S. A., & Kulkarni, B. D. (2020). Valorization of cow urine and dung: A model biorefnery. *Waste and Biomass Valorization*, 11, 1191-1204. https://doi.org/10.1007/s12649-018-0406-7
- Miranda, R. M., Dias, D. C. F. S., Picoli, E. A. T., Silva, P. P., & Nascimento, W. M. (2017). Physiological quality, anatomy and histochemistry during the development of carrot seeds (*Daucus carota L.*). *Ciência e* Agrotecnologia, 41(2), 169-180. https://doi.org/10.1590/1413-70542017412009216
- Nápoles, F. A. M., Souza, J. T. A., Azevedo, C. A. V., Souza, G. A. V. S., Oresca, D., Josemo, S., & Oliveira, C. (2018). Jatropha production in function of organic fertilization with cassava water and cow urine. *Revista de Agricultura Neotropical*, 5(2), 6-12. https://doi.org/10.32404/rean.v5i2.1855
- Neto, A. F., Oliveira, S. B., Lima, M. S., Amorim, M. R., & Figueiredo, R. M. (2011). Influência de composto orgânico nas características físico-químicas de cenoura "Brasília" no município de Petrolina (PE). *Revista Semiárido*, 1(1), 3-9. https://doi.org/10.31416/rsdv.v1i1.204
- Nwite, J. N. (2015). Effect of different urine sources on soil chemical properties and maize yield in abakaliki, southeastern Nigeria. *International Journal of Advance Agricultural Research*, *3*, 31-36.
- Oliveira, A. P., Paes, R. A., Souza, A. P., Dornelas, C. S. M., & Silva, R. A. (2004). Produção de pimentão em função da concentração de urina de vaca aplicada via foliar e da adubação com NPK. *Agropecuária Técnica*, 25(1),37-43.
- Oliveira, N. L. C. (2007). *Utilização de urina de vaca na produção orgânica de alface*. Viçosa, MG: Universidade Federal de Viçosa.
- Oliveira, N. L. C., Puiatti, M., Santos, R. H. S., Cecon, P. R., & Bhering, A. S. (2015). Efeito da urina de vaca no estado nutricional da alface. *Revista Ceres*, 57(4), 506-515. https://doi.org/10.1590/S0034-737X2010 000400011
- Pradhan, S. S., Verma, S., Kumari, S., & Singh, Y. (2018). Bio-efficacy of cow urine on crop production: A review. *International Journal of Chemical Studies*, 6(3), 298-301.
- Pradhan, S. S., Verma, S., Kumari, S., & Singh, Y. (2018). Bio-efficacy of cow urine on crop production: A review. *International Journal of Chemical Studies*, 6(3), 298-301.
- Sadhukhan, R., Bohra, J. S., & Choudhury, S. (2018). Effect of fertility levels and cow urine foliar spray on growth and yield of wheat. *International Journal of Current Microbiology and Applied Sciences*, 7(3), 907-912. https://doi.org/10.20546/ijcmas.2018.703.106
- Sharma, G., Sahu, N. P., & Shukla, N. (2017). Effect of bio-organic and inorganic nutrient sources on growth and flower production of african marigold. *Horticulturae*, 3(11), 1-5. https://doi.org/10.3390/horticulturae 3010011

- Souza, B. N., Costa, Silva, I. J., Ferreira, D. M., Fonseca, W. B. J., Teodoro, F. M., & Cunha, L. M. V. (2011). Parâmetros produtivos da cebola cristal white wax (para conserva) no sistema integrado Mandalla sob doses de urina de vaca em lactação. *Cadernos de Agroecologia, 6*(2), 13-21.
- Testani, E., Montemurro, F., Ciaccia, C., & Diacono, M. (2020). Agroecological practices for organic lettuce: Effect on yield, nitrogen status and nitrogen utilization efficiency. *Biological Agriculture & Horticulture*, 36(2), 84-95. https://doi.org/10.1080/01448765.2019.1689531
- Verma, S., Singh, A., Pradhan, S. S., Singh, J. P., & Verma, S. K. (2018). Effects of organic formulations and synthetic fertilizer On the performance of pigeonpea in eastern Region of uttar pradesh. *Bangladesh Journal* of Botany, 47(3), 467-471. https://doi.org/10.3329/bjb.v47i3.38713

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