Intercropping with *Tropaeolum majus* and fertilization with chicken manure on yield of *Allophylus edulis*¹

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10.1590/0034-737X201966050004

ABSTRACT

Proper crop management is essential for preservation of medicinal plants of the Cerrado. There is no report of *ex-situ* cultivation of *Allophylus edulis* (vacum), which is used for its antimicrobial and antioxidant activities, in intercropping with *Tropaeolum majus* (nasturtium). Therefore, the aim of this study was to evaluate the effects of the intercropping with nasturtium and fertilization with chicken manure on yield of vacum. The experiment consisted of six treatments: monocrop vacum without chicken manure; monocrop vacum with hicken manure; monocrop nasturtium without chicken manure; monocrop nasturtium with chicken manure; vacum intercropped with nasturtium without chicken manure and vacum intercropped with nasturtium with chicken manure. The crop cycle of vacum took 540 days after transplanting and, during this period, nasturtium was cultivated in two cycles, in 2017 and 2018. Vacum showed greater height growth and production of fresh and dry masses of leaves in single cultivation with chicken manure fertilization. Nasturtium showed greater growth and flower production in monocrop cultivation with chicken manure fertiliazation, in the first cycle. The intercropping vacum with nasturtium was effective, showing Equivalent Area Ratio (EAR) of 1.16 and 1.18 without and with chicken manure, respectively.

Keywords: nasturtium; vacum; organic waste; plant arrangement.

INTRODUCTION

Proper crop management is essential for preservation of native medicinal plants. Cerrado is one of the biomes that are rich in species of economic interest. It is the second largest Brazilian vegetation unit and is considered the richest in plant species among the world’s Savannas. Hence, the risk of biodiversity loss due to intensification of farming activities, spread of exotic species, deforestation, burnings, and degradation of natural resources (Fernandes *et al*., 2016).

The exploitation of medicinal plant genetic resources is associated with extractive activities, making them more susceptible to extinction (Fernandes *et al*., 2016; Jeromini *et al*., 2018). Therefore, the need for studies regarding cultivation practices, including intercropping, with advantages such as proper use of natural resources, harvest at different times, maximization of space with species of different habits, use of organic waste, greater growth and yield of the species (Brito *et al*., 2017).

*Allophylus edulis* (A. St.-Hil., A. Juss. Cambess.) Hieron. ex Niederl (vacum, Sapindaceae) is native to Brazil, occurring in the Cerrado of Mato Grosso do Sul and Minas Gerais. Its leaves have medicinal properties against throat inflammations (Trevizan *et al*., 2016), diarrhea (Umeo *et al*., 2011), and antimicrobial and antioxidant activities due to the presence of phenolic compounds such as alkaloids, flavonoids, and essential oils (steroid and...
Triterpenoid) in its chemical composition (Tirloni et al., 2015). Economically, it is used as wood in carpentry and afforestation of cities; the ripe fruits are sweet and eaten by birds and other animals (Umeo et al., 2011; Trevizan et al., 2016).

*Tropaeolum majus* L. (nasturtium, Tropaeolaceae) has potential to be intercropped with vacum. Nasturtium is an edible plant with medicinal properties against cardiovascular disorders (Jakubczyk et al., 2018), urinary tract infections, antiseptic and expectorant activities (Melo et al., 2018). As a vegetable, the whole aerial part is used for human consumption, with leaves and flowers being sources of vitamin C and mineral salts (Jakubczyk et al., 2018).

Literature reviews have shown that there were no studies regarding the intercropping of vacum with nasturtium or addressing its growth and yield, indicating the importance of preserving this species native to the Cerrado, decreasing the risk of extinction, fighting indiscriminate use, and using proper cultivation management (Fernandes et al., 2016; Oliveira et al., 2015). Intercropping of medicinal plants has been studied by some authors (Brito et al., 2017; Moraes et al., 2007); however, investigations with species native to Cerrado are still lacking, making researches that generate knowledge to provide rational use of natural resources necessary.

In addition to intercropping, the use of organic waste as chicken manure, which is a primary source of nutrients, can contribute to reduce reliance on agricultural inputs, as well as to provide a sustainable destination for these materials (Torales et al., 2014; Rogeri et al., 2015). Organic manure can increase medicinal plant production by promoting slow and gradual release of nutrients to the soil over time and improving physical, chemical, and biological attributes of the soil such as reduction of soil density, increase in soil porosity, and increase in soil-water retention (Costa et al., 2009), increase in cation exchange capacity, increase levels of P, K, Ca, Mg, Cu and Zn (Rogeri et al., 2015), and increase soil microbial respiration (Andrade et al., 2015).

The beneficial effect of organic wastes to plants has been demonstrated by Bonamigo et al. (2016), who found that the substrate composed of soil, sand and poultry manure favored the growth and quality of seedlings of *Tocoyena formosa* (Cham. & Schltdl.) K. Schum. Bortolini et al. (2017) reported that poultry manure with sewage sludge improved height and diameter growth rates of seedlings of *Cedrela fissilis* and *Anadenanthera macrocarpa* (Benth). Brenan. Sangalli et al. (2004) found that chicken manure added of nitrogen enhanced plant growth and flower production in *Tropaeolum majus* L. However, limited studies are available on intercropping with native medicinal plants and animal manure fertilization. Thus, the aim of this study was to assess the yield of vacum intercropped with nasturtium and fertilization with chicken manure.

**MATERIAL AND METHODS**

The experiment was performed in the Medicinal Plant Garden (22°13'16"S and 54°17'01"W, 452m altitude) of the Universidade Federal da Grande Dourados, Dourados-MS, from July 2017 to October 2018. The soil is classified as Dystroferric Red Latosol (Embrapa, 2013) and the climate as mesothermal humid, type Am, tropical, with rainy summers. Temperature range from 20 to 24 °C and annual rainfall from 1250 to 1500 mm (Alvares et al., 2013). Temperatures and rainfall during cultivation are shown in Figure 1.

Vacum and nasturtium cv. ‘Jewel’ were studied in monocrop and intercrop systems, with or without addition of of 15 t ha⁻¹ of chicken manure to the soil. The experiment consisted of six treatments as follows: monocrop vacum without chicken manure; monocrop vacum with chicken manure; monocrop nasturtium without chicken manure; monocrop nasturtium with chicken manure; vacum intercropped with nasturtium without chicken manure and vacum intercropped with nasturtium with chicken manure. The experiment was arranged in a randomized block design with four replicates. Each plot had an area of 2.5 m² (1 m wide and 2.5 m long), with one row of vacum with five plants spaced 0.50 m, with population of 20.000 plants ha⁻¹, and two rows of nasturtium with ten plants each spaced 0.50 m between rows and 0.25 cm between plants, with population of 75.000 plants ha⁻¹. The same spacings were used in the plots with intercrop, and the total field experiment size was 75 m².

The species were propagated in polystyrene trays with 128 cells, using Bioplant® substrate, in protected environment with 50% shading. Vacuum seeds were extracted from fruits randomly harvested (Registration SISGEN number A9CDAAE) from plants of natural populations in Dourados-MS (22°08’23.24"S and 55°08’16.84"W, 487 m altitude).

Seeds of a commercial variety of nasturtium were purchased from Isla® Sementes. When the vacuum seedlings reached 15 cm in height, they were transferred to tubes and transplanted to the field with about 50 cm in height, at one year of age. Nasturtium was cultivated in two cycles, in 2017 and 2018. In the first cycle, 2017, the seedlings were transplanted 25 days after sowing (DAS) on the same day that vacum was transplanted, and in the second cycle, 2018, at 190 days after vacuum transplanting, both with about 12 cm in height.

The planting area was prepared by plowing and harrowing and seedbeds were prepared with a rotary cultivator. Chicken manure was added to the respective plots,
Intercropping with *Tropaeolum majus* and fertilization with chicken manure on yield... Intercropping with *Tropaeolum majus* and fertilization with chicken manure on yield... at 0.0-0.20 m depth, using a rotary cultivator, on the day after the transplanting. Chicken manure contained 260.0 g kg\(^{-1}\) C\(_{\text{org}}\); 15.36 g kg\(^{-1}\) P\(_{\text{tot}}\); 20.0 g kg\(^{-1}\) K\(_{\text{tot}}\); 23.9 g kg\(^{-1}\) N\(_{\text{tot}}\); and 447.0 g kg\(^{-1}\) MO, and C/N=10.87. Sprinkler irrigation was performed to maintain soil moisture at 70% field capacity. Weeding was carried out by hand when necessary.

The characteristics assessed for the vacum plants were as follows: collar diameter and height measured every 30 days, from 30 to 540 days after transplanting - DAT. Fruits were harvested weekly, in 2017 (90 to 120 DAT) and 2018 (420 to 510 DAT), and after weighing, the production was summed up to determine the total production of each year. At 540 DAT, plants were harvested by cutting at 0.50 m above the collar, fresh and dry masses of leaves and stems weighed, and leaf area assessed using a leaf area integrator LI 3100 (LI-COR, Lincoln, Nebraska, USA).

After weighing, the leaves were dried to constant mass in a forced air circulation oven at 65 °C ± 5 °C to determine the dry mass. Then, the material was ground in a Willey type mill for further analysis of macro and micronutrient content, according to Malavolta (2006). Soil samples from all treatments were collected to perform the characterization of the chemical attributes. The nasturtium plants had their height measured during the cultivation cycles and flowers in anthesis were harvested for assessment of number and fresh and dry masses.

The following physiological parameters of vacuum and nasturtium plants were assessed: transpiration (\(E\)) and photosynthetic (\(A\)) rate, stomatal conductance (\(g_s\)), intercellular CO\(_2\) concentration (\(C_i\)), instantaneous water use efficiency (\(A E^{-1} - WUE\)), instantaneous carboxylation (\(A C_i^{-1} - ICE\)), and intrinsic water use efficiency (\(A G_s^{-1} - WUEi\), \(\text{mol mol}^{-1}\)). The parameters were assessed in fully expanded leaves of vacuum plants at 30, 60, 90, 120, 420, 450, 480, and 510 DAT and in nasturtium leaves at 30, 60, 90, and 120 DAT in each cycle, in the morning, using an infrared gas analyzer - IRGA (LI-Cor, model LI-6400, ADC System) with 300 mL min\(^{-1}\) airflow and a 995 µmol m\(^{-2}\) s\(^{-1}\) light source. The chlorophyll index (CI) was determined using a portable chlorophyllometer (ClorofiLOG CFL 1030 Falker).

The efficiency of the intercropping was assessed using the Equivalent Area Ratio (EAR) (Caetano *et al.*, 1999), with the equation: EAR = (C\(_v\)/S\(_v\)) + (C\(_n\)/S\(_n\)); where C\(_v\) and C\(_n\) = vacuum and nasturtium productions in the intercropping and S\(_v\) and S\(_n\) = vacuum and nasturtium productions in monocropping.

Data were subjected to analysis of variance by the F test, and means were compared by the Tukey's test (p \(\leq\) 0.05). Data assessed during the cultivation cycles were analyzed as plots subdivided in time, subjected to analysis of variance by the F test, and regression models were adjusted at 5%. Principal component analysis was used to understand the relationship among intercrop, monocrop, and chicken manure (Sneath & Sokal, 1973).

### RESULTS

The soil in the intercropping system with addition of chicken manure had the highest contents of P, K, SB, V% and lowest Al, at the end of the crop cycle. Monocrop vacuum with addition of chicken manure had higher contents of K, OM and SB; and monocrop nasturtium with chicken manure had higher contents of OM and Zn. The contents of Ca, Mg, Cu, Mn, Fe, pH, H+Al, and CEC were not different among the factors under study (Table 1).

![Figure 1: Maximum and minimum 10-day temperature and rainfall averages, from July 10, 2017 to October 27, 2018. (Source: clima.cpaob.embrapa.br)](image-url)
Table 1: Chemical attributes of soil before and at the end of the experiment, regarding monocrop and intercrop systems, with and without addition of chicken manure

| Attributes   | Initial | With chicken manure | Without chicken manure | C.V. (%) |
|--------------|---------|---------------------|-------------------------|----------|
|              |         | Vacuum Nasturtium   | Consortium              |          |
|              |         |                     |                         |          |
| P (mg dm⁻³)  | 14.33   | 51.17±13.65ab       | 51.82±23.78ab           | 35.32a   |
| K (mmolc dm⁻³) | 5.40    | 2.10±0.09a          | 1.70±0.07ab             | 9.01b    |
| Ca (mmolc dm⁻³) | 35.10 | 75.00±0.54a         | 75.00±0.85a             | 14.58a   |
| Mg (mmolc dm⁻³) | 17.30 | 22.50±0.16a         | 25.00±0.21a             | 20.00±0.30a |
| Cu (mg dm⁻³)  | -       | 13.25±0.37a         | 13.00±0.85a             | 12.50±1.09a |
| Mn (mg dm⁻³)  | -       | 92.00±2.93a         | 86.75±9.43a             | 82.00±7.59a |
| Fe (mg dm⁻³)  | -       | 123.75±15.55a       | 123.00±11.48a           | 139.25±24.01a |
| Zn (mg dm⁻³)  | -       | 4.75±0.84a          | 5.00±1.24a              | 3.50±0.53a |
| OM (g dm⁻³)   | 27.31   | 33.75±0.62a         | 31.75±2.97a             | 29.25±2.53a |
| pH            | 5.40    | 5.25±0.12a          | 5.35±0.07a              | 5.11±0.12a |
| Al (mmolc dm⁻³) | 1.00 | 0.90±0.06ab         | 0.60±0.06ab             | 2.10±0.06a |
| H+Al (mmolc dm⁻³) | 57.60 | 34.70±0.23a         | 32.90±0.10a             | 33.50±0.06a |
| SB (mmolc dm⁻³) | 5.80 | 2.10±0.09a          | 1.60±0.07ab             | 0.90±0.01ab |
| CEC (mmolc dm⁻³) | 115.40 | 36.60±0.30a         | 34.10±0.14a             | 39.90±0.77a |
| V%            | 5.09    | 5.40±2.47ab         | 4.67±1.86ab             | 2.60±0.33b |

Means followed by the same letters in the rows are not significantly different by the Tukey’s test at the level of 5% of probability (p > 0.05). Content of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn), iron (Fe), zinc (Zn), organic matter (OM), hydrogen potential (pH), aluminum (Al), potential acidity (H + Al), sum of bases (SB), cation exchange capacity (CEC), and base saturation (V%). C.V. - coefficient of variation.
leaves of plants in intercropping without chicken manure. The contents of Ca and Mn were higher in monocropping with addition of chicken manure, differing only from the intercropping without addition of chicken manure. Foliar contents of K, Mg, Cu, and Zn were not different among the factors under study in monocropping (Table 2). Intercropped nasturtium with addition of chicken manure had higher foliar content of N and K (Table 2).

Over the cycle, the growth in height of vacuum plants was linear, being greater in monocropping, with the maximum height of 147.17 cm at 540 DAT (Figure 2A). The greatest stem diameter of vacuum plants was achieved in monocropping, with maximum of 21.06 mm at 540 DAT (Figure 2B), while with the addition of chicken manure, the maximum diameter was 20.66 mm at 540 DAT (Figure 2C).

The maximum heights of nasturtium plants occurred in monocropping, with maximum of 21.06 mm at 540 DAT (Figure 2B), while with the addition of chicken manure, the maximum of 20.66 mm at 540 DAT (Figure 2C).

The physiological parameters of vacuum plants showed higher photosynthetic rate (A), intercellular CO$_2$ concentration (Ci), instantaneous water use efficiency (WUE), intrinsic water use efficiency (WUEi), and indexes of Chlorophyll a and total with addition of chicken manure, regardless of the intercropping. On the other hand, the transpiration rate (E) was greater in the intercropping without addition of chicken manure. The stomatal conductance (gs), instantaneous carboxylation efficiency (ICE), and Chlorophyll b index showed no difference among the factors under study (Table 3). The indexes of Chlorophyll a, b, and total in nasturtium plants were higher with addition of chicken manure, regardless of the intercropping and cycles (Table 3).

Regarding the physiological parameters of nasturtium plants, the lowest intercellular CO$_2$ concentration (Ci) (280 µmol mol$^{-1}$; w=384.656250-2.678021*x+0.017135*x$^2$; R$^2$=0.88) was recorded in the 2017 cycle at 78 DAT. The stomatal conductance (gs) (from 0.375 to 0.185 µmol mol$^{-1}$; w=0.3957-0.0018*x; R$^2$=0.75) decreased over the cultivation cycle, regardless of the intercropping and addition of chicken manure.

In the second crop cycle of nasturtium, the maximum stomatal conductance (gs) of 0.30 µmol mol$^{-1}$ (w=-0.0059+0.0082*x-0.000066*x$^2$; R$^2$=0.77) was recorded at 540 DAT and the maximum instantaneous carboxilation efficiency (ICE) of 0.03 µmol m$^{-2}$ s$^{-1}$ (w=0.0004+0.0012*x-0.000009*x$^2$; R$^2$=0.95) at 66 DAT. The maximum transpiration rate (E) of 5.07 mmol m$^{-2}$ s$^{-1}$ (w=2.802187+0.084430*x-0.000784*x$^2$; R$^2$=0.90) was recorded at 53 DAT and the maximum photosynthetic rate (A) of 11.48 µmol m$^{-2}$ s$^{-1}$ (w=1.4632+0.3102*x-0.0024*x$^2$; R$^2$=0.99), at 64 DAT, regardless of the intercropping and addition of chicken manure.

### Table 2: Contents of macro and micronutrients in leaves of vacuum and nasturtium in monocropping and intercropping systems, with and without addition of chicken manure

| Nutrient | With chicken manure | Without chicken manure | C. V. (%) |
|----------|---------------------|------------------------|----------|
|          | Vacuum              | Intercrop              |          |          |          |          |
| N (g kg$^{-1}$) | 138.60±1.14 a       | 136.50±8.66 a          | 51.10±14.56 b | 43.75±10.19 b | 40.80 |
| P (g kg$^{-1}$)  | 3.74±0.70 ab        | 4.07±0.64 ab           | 2.95±0.23 b | 4.25±0.48 a | 15.31 |
| K (g kg$^{-1}$)  | 1.83±0.42 a         | 1.92±0.58 a            | 1.54±0.30 a | 1.82±0.42 a | 27.15 |
| Ca (g kg$^{-1}$) | 11.57±1.28 a        | 11.18±1.21 ab          | 9.93±0.76 ab | 9.25±0.76 b | 9.84 |
| Mg (g kg$^{-1}$) | 3.68±0.31 a         | 3.59±0.40 a            | 3.53±0.31 a | 3.59±0.26 a | 5.10 |
| Cu (mg kg$^{-1}$)| 4.11±0.28 a         | 4.03±0.52 a            | 3.54±0.35 a | 3.76±0.44 a | 9.38 |
| Mn (mg kg$^{-1}$)| 96.89±8.51 a        | 86.76±7.05 ab          | 84.89±1.67 ab | 69.87±3.31 b | 9.39 |
| Zn (mg kg$^{-1}$)| 20.67±7.24 a        | 22.45±12.62 a          | 14.00±6.27 a | 23.83±5.45 a | 42.62 |

Means followed by the same letters in the rows are not significantly different by the Tukey’s test at the level of 5% of probability (p > 0.05). Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn), and zinc (Zn). C.V. - coefficient of variation.

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Rev. Ceres, Viçosa, v. 66, n.5, p. 351-362, sep/oct, 2019
The maximum intercellular CO$_2$ concentration (\(C_i\)) (290.84 \(\mu\)mol mol$^{-1}$; \(w=254.2254+0.5715x-0.0032x^2; \ R^2=0.76\)) and reduction in instantaneous carboxylation efficiency (ICE) (0.026 mol m$^{-2}$ s$^{-1}$; \(w=0.0331-0.0002x+0.000001x^2; \ R^2=0.79\)) were found at 210 DAT, regardless of the intercropping and addition of chicken manure, since at low concentrations of CO$_2$ photosynthetic rates are limited (Taiz et al., 2017).

Fresh and dry masses of leaves and leaf area of vacum were higher in monocropping with addition of chicken manure, except for fresh mass of leaves that did not differ with or without addition of chicken manure. The fresh and dry mass of stems showed no difference between treatments (Table 4). No difference was found for fruit production of vacum as a function of the treatments, with mean fresh mass of 4.29 g plant$^{-1}$ and dry mass of 1.25 g plant$^{-1}$, probably due to the short period of assessment, since the species is perennial.

The flower production of nasturtium was maximum with addition of chicken manure in the first cycle, regardless of the intercropping, producing the greatest number (113.54 plant$^{-1}$), fresh mass (69.21 g plant$^{-1}$), and dry mass (5.17 g

![Figure 2](image-url)  
**Figure 2**: Plant height (A) and stem diameter of vacum as a function of days after transplanting, in intercropping (B) and addition of chicken manure to the soil (C).

![Figure 3](image-url)  
**Figure 3**: Height (cm) of nasturtium as a function of addition of chicken manure to the soil, in 2017 (A) and as a function of intercropping, in 2018 (B).
Intercropping with *Tropaeolum majus* and fertilization with chicken manure on yield...

No difference was found between treatments in the second cycle.

The principal component analysis (PCA) related the characteristics analyzed of vacum and nasturtium plants. Axis 1 explained 53.30% of data variation and axis 2 explained 42.02%, totaling 95.32% of total data variability in vacum plants (Figure 4A). Regarding nasturtium plants, axis 1 explained 58.93% and axis 2 explained 33.10%, totaling 92.03% of total data variability (Figure 4B).

### Table 3: Physiological parameters and chlorophyll $a$, $b$, and total indexes in vacum and nasturtium as a function of monocropping and intercropping with nasturtium, with and without chicken manure

| Parameters                        | With chicken manure                          | Without chicken manure                          | C. V. (%) |
|-----------------------------------|----------------------------------------------|-----------------------------------------------|-----------|
|                                   | Monocrop | Intercrop | Monocrop | Intercrop |               |
| $Ci$ (µmol mol⁻¹)                 |          |           |          |           |               |
|                                   | 270.30±33.40 a | 261.09±27.70 ab | 242.33±23.55 b | 252.40±26.02 ab | 10.53 |
| $E$ (mmol m⁻² s⁻¹)                | 3.22±0.92 b | 3.67±1.16 ab | 3.43±1.47 ab | 4.27±0.92 a | 30.94 |
| $Gs$ (mol m⁻² s⁻¹)                | 10.57±1.95 a | 9.73±2.42 a | 7.82±1.38 b | 7.85±1.97 b | 24.46 |
| $WUE$ (mol CO₂ mol⁻¹ H₂O)         | 3.45±0.73 a | 2.72±0.36 ab | 2.75±0.36 ab | 2.31±0.50 b | 36.27 |
| $ICE$ (mol m⁻² s⁻¹)               | 86.94±27.14 a | 83.00±11.15 ab | 75.53±14.50 bc | 73.32±13.64 c | 25.69 |
| $WUEi$ (µmol mol⁻¹)               | 34.26±1.49 a | 34.24±0.72 a | 34.24±2.45 a | 34.26±3.20 a | 15.26 |
| Chlorophyll $a$                   | 31.67±1.68 a | 31.18±2.09 a | 27.64±2.84 b | 26.61±2.14 b | 9.32 |
| Chlorophyll $b$ 2018             | 12.22±0.69 a | 10.39±0.82 ab | 8.72±0.45 b | 7.91±0.35 c | 21.75 |
| Chlorophyll total                 | 43.92±2.12 a | 41.57±2.87 a | 36.43±1.12 b | 34.59±2.16 b | 10.09 |
| Chlorophyll $a$                   | 30.66±4.46 a | 30.72±3.47 a | 24.02±1.45 b | 25.45±1.66 b | 14.40 |
| Chlorophyll $b$ 2019             | 11.83±1.93 a | 12.56±0.77 a | 8.48±1.12 b | 8.66±0.53 b | 18.64 |
| Chlorophyll total                 | 42.50±6.38 a | 43.24±2.45 a | 32.50±1.57 b | 34.10±2.12 b | 14.57 |

Means followed by the same letters in the rows are not significantly different by the Tukey’s test at the level of 5% of probability (p > 0.05). $Ci$ - intercellular CO2 concentration, $E$ - transpiration rate, $Gs$ - stomatal conductance, $WUE$ - instantaneous water use efficiency, $ICE$ - instantaneous carboxylation efficiency, $WUEi$ - intrinsic water use efficiency. C.V. - coefficient of variation.

### Table 4: Effect of crop system and addition of chicken manure to the soil on the production fresh (FML) and dry (DML) mass of leaves and stems (FMS and DMS) and leaf area (LA) of vacuum at 540 DAT and nasturtium at 140 DAT in 2018

| Variables | With chicken manure | Without chicken manure | C. V. (%) |
|-----------|---------------------|------------------------|-----------|
|           | Monocrop | Intercrop | Monocrop | Intercrop |               |
| FML (g plant⁻¹) | 247.02±25.98 a | 142.86±5.34 ab | 235.32±18.35 a | 117.38±7.57 b | 36.29 |
| DML (g plant⁻¹) | 79.32±9.23 a | 39.54±2.97 ab | 68.65±5.30 ab | 31.83±2.14 b | 47.65 |
| FMS (g plant⁻¹) | 186.25±30.22 a | 12.46±9.57 a | 102.95±12.50 a | 120.95±12.50 a | 63.69 |
| DMS (g plant⁻¹) | 104.76±14.39 a | 72.70±4.59 a | 99.33±9.48 a | 72.93±5.77 a | 51.10 |
| LA (cm² plant⁻¹) | 8318.04±80.22 a | 120.46±9.57 a | 178.51±21.16 a | 120.95±12.50 a | 63.69 |

Means followed by the same letters in the rows are not significantly different by the Tukey’s test at the level of 5% of probability (p > 0.05). C.V. - coefficient of variation.
The second group (Figure 4A) comprised the intercropping of vacum with nasturtium with addition of chicken manure, which was explained by the variables SB, K-s, V%, P-s, Cu-v, Zn-v, pH, and P-v. The third group comprised the monocropping of vacuum without addition of chicken manure, which was explained by H+Al and CEC, and the fourth group corresponded to the intercropping without addition of chicken manure, which was explained by F-s and Cl.

Characteristics of nasturtium were separated into four groups. The first group comprised the monocropping with chicken manure, which was explained by FML, FM FL, DM FL, LA, E, WUE, Mn-v, Cu-v, chlorophyll a, b, and total (Figure 4B).

The second group comprised the intercropping of nasturtium with addition of chicken manure, which was explained by SB, K-s, V%, Mg-v, Ca-v, and Cl. The third and fourth groups corresponded to monocropping of

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**Figure 4**: Principal component analysis (PCA) of variables related to growth, production, physiological parameters, and chemical characteristics of plant and soil material as a function of monocrop and intercrop systems, with and without addition of chicken manure to the soil. Ca-p = plant calcium; P-p = plant phosphorus; K-s = soil potassium; Fe-s = soil iron; Ca-s = soil calcium; Zn-p = plant zinc, Cu-p = plant copper; K-p = plant potassium, Mn-p = plant manganese.
nasturtium and intercropping without addition of chicken manure and were explained by Al and WUE and CEC, respectively (Figure 4B).

Intercropping efficiency was calculated according to values of Equivalent Area Ratio (EAR) as 1.16 and 1.18 for the treatments without and with chicken manure, respectively, and therefore it was considered efficient.

**DISCUSSION**

The intercropping and the addition of chicken manure favored P adsorption by soil, due to its anionic character, since the sites electrostatically attract cations such as Ca, Fe, and Al, which induce P retention (Sá et al., 2017). According to the classification by Raij (2017), the P content in the soil found in this study is considered high (> 41 mmol dm⁻³). These results corroborate the study of Rodrigues et al. (2016) who evaluated the intercropping of *Acacia mangium* Willd., native to Brazil, with *Pueraria phaseoloides* (Roxb.) Benth, forage Fabaceae, and reported improvement of soil chemical attributes.

Species native to the Cerrado such as vacum occur in areas of acid and low fertility soils, therefore, they are adapted to the soil conditions where this study was performed (Knkapik et al., 2005; Umeo et al., 2011). Nevertheless, the OM content was satisfactory for the effectiveness of CEC, supplying of nutrients such as P and K, maintenance of pH, and reduction of Al content. This is a consequence of the mineralization process that transforms organic material into organic substances by increasing the negative charges of the soil and raising the pH (Sá et al., 2017). In in this study, the nutrient contents in vacum were within the range considered suitable, according to Raij (2017), for crop development: N (40-60 g kg⁻¹), P (3-7 g kg⁻¹), K (1-5 g kg⁻¹), Ca (4-40 g kg⁻¹), Cu (5-30 mg dm⁻³), Mn (20-300 mg dm⁻³), and Zn (27-150 mg dm⁻³).

The treatment intercropping without chicken manure resulted in greater foliar P content in vacum plants, probably because it is an undemanding native species adapted to use P more efficiently. The concentration of P in the soil solution is low which is considered a limiting factor to more demanding plants, like nasturtium, due to the strong adsorption to iron oxides and aluminum from the clay fraction (Rodrigues et al., 2016; Raij, 2017). P stimulates root growth and enhances reproductive growth, as it is a constituent of the cytoplasmic and nuclear proteins and has important role in the carbohydrates metabolism and energy transfers (Taiz et al., 2017).

The importance of high foliar K content in nasturtium is because of its role in cellular osmotic regulation, activation of enzymes in the photosynthesis, and respiration processes, consequently, leading to a greater growth rate and flower maturation (Taiz et al., 2017).

The higher nutrient content in leaf dry mass of nasturtium, in relation to vacum, indicates that this species requires higher amounts of these nutrients for leaf formation, as well as to meet the metabolic demands of flowers produced along the cultivation cycle (Jakubczyk et al., 2018).

Vacum had greater height and diameter of stem in monocropping due to the lack of competition with nasturtium. The height values in these treatments were greater than those reported by Knkapik et al. (2005) for the same species propagated by seeds and cultivated with mineral fertilization; they observed maximum height of 8.02 cm at 120 DAT. They also found that the plant was not responsive to fertilization, indicating lower demand for nutrients and slow growth over the years. The increase in stem diameter with addition of chicken manure is result from the effects on greater nutrient availability as well as improvements in physical, chemical, and microbiological characteristics of soil (Kiehl, 1985).

The fact that chicken manure contributed to growth of nasturtium corroborates the findings of Sangalli et al. (2004), who reported that the improved nutrient availability, infiltration, and water retention resulting from the use of organic waste contributed to the growth of nasturtium, mainly at the beginning of the crop cycle. In addition, Moraes et al. (2007) observed mean height of 21.05 cm in in full bloom nasturtiums at 70 DAT.

Nasturtiums are more demanding in nutritional terms than vacum plants (Jakubczyk et al., 2018); hence, the greater growth rate in monocropping is a result of less competition for nutrients, light, water, oxygen, and others. The lower growth in 2018 is due to high rainfall and high temperatures, which reduced plant growth, since the species is adapted to mild temperatures, with mean of 21.61 ºC.

Gas exchange in vacum was greater by the effect of chicken manure on soil chemical properties, increasing the contents of P and Mg, which act as cofactors in enzymes of energetic metabolism, in the chlorophyll molecule, and conversion of light energy into ATP (Oliveira et al., 2018). Moreover, the nutrients provided by chicken manure can contribute to the transport and absorption of ions and speed of enzymatic reactions of the Krebs cycle, resulting in increased production of metabolic energy and increasing the synthesis of nucleic acids (Taiz et al., 2017).

As a consequence, there was a significant increase in shoot growth of vacum, favoring the exposure of leaves to direct sunlight and photosynthesates production (Taiz et al., 2017). The increase in the values of chlorophyll a and total indices in vacum are due to the beneficial effects of chicken manure on nutrient availability, namely N, Mg, P, and micronutrients, which are related to photosynthesis and constituents of chlorophyll molecule (Armond et al., 2017).
2016). Studies assessing chlorophyll index with addition of organic fertilizers resulted in favorable effects, as reported by Sales et al. (2018) on Schinus terebinthifolius fertilized with tannery sludge, waste from coffee roasting industry, and cow manure.

Water use efficiency (WUEi) and instantaneous water use efficiency (WUE) were greater in vacuum because of the species ability to regulate gas exchange, by decreasing stomatal conductance and transpiration more than CO₂ assimilation, saving water for each CO₂ molecule assimilated (Taiz et al., 2017).

Greater evapotranspiration and photorespiration and lower efficiency of Rubisco in vacuum plants without chicken manure led to lower growth rate, since the reduction in mesophyll metabolism limits CO₂ absorption in the chloroplasts (Taiz et al., 2017).

The concentrations of chlorophyll a, b and total were higher in nasturtium with addition of chicken manure. This result is due to greater foliar N content, which is important for photosynthesis and plant growth as a constituent of the chlorophyll molecule (Armond et al., 2016). The chlorophyll b index enables the capture of larger amount of incident light and channel it to the sites of action of photosystems, thus providing greater ATP and NADPH formation (Sales et al., 2018).

The lowest intercellular CO₂ concentration in nasturtium, in the 2017 cycle, stimulated stomatal opening, allowing entry of CO₂ to support high photosynthetic rates and instantaneous water use efficiency. The decrease in stomatal conductance along the crop cycle, regardless of the intercropping and addition of chicken manure, led to senescence and leaf fall, emergence of structures, and assimilating tissues (Taiz et al., 2017), therefore causing reduction in height and plant productivity.

When the leaves began the senescence process, the photosynthetic rates began to decrease due to chlorophyll degradation (Taiz et al., 2017), with a progressive reduction of gas exchange, with minimum values at 120 DAT; thereby the reduction in plant height, which was more intense in the second cultivation cycle.

The photosynthetic rate (A) during the second cycle of nasturtium was higher in the intercropping with chicken manure because of the nutrient release to the soil, improving chemical conditions and, consequently, the gas exchanges that contribute to plant growth (Andrade et al., 2015; Sá et al., 2017). Oliveira et al. (2018) reported similar results, with increase in A, E and WUE in plants of Lycopersicon lycopersicum var. Cerasiforme cultivated with chicken manure.

Production of leaves in monocropped vacuum was greater due to better adaptation and less competition between plants for water, nutrients, light, and CO₂, together with the addition of chicken manure, contributing to a higher organic matter content. C-related nutrients, like N and P, are released from microbial cells to the soil as mineral, making them available for absorption by plants, thus, contributing to growth and productivity (Malavolta, 2006; Andrade et al., 2015; Jakubczyk et al., 2018).

Different results were reported by Knipik et al. (2005), studying the effect of mineral fertilization [(NH₄)₂SO₄, P₂O₅ and KCl] on vacuum. The authors found no response regarding fresh mass of leaf and stem and concluded that species native to Cerrado are undemanding for fertilization.

The positive response to organic fertilization in this study results from the different conditions of soil, climate, and others, it is, therefore, important to produce more leaf mass and consequently more secondary metabolites (Knipik et al., 2005; Torales et al., 2014; Trevizan et al., 2016).

Agronomic practices that improve vacuum yield are key to phytotherapy, because the essential oil is produced in the leaves. Vacuum oil has viridiflorol (30.88%) as main constituent. It is used against Mycobacterium tuberculosis, and as a natural antiinflammatory, antimycobacterial and antioxidant (Trevizan et al., 2016).

Studies performed by Tirloni et al. (2015), Trevizan et al. (2016) and Umeo et al. (2011) proved the benefits of flavonoids and phenolic compounds from essential oil of vacuum, highlighting the importance of cropping systems to increase productivity, since the exploitation of genetic resources and extractive harvest of Cerrado can lead to reduction of natural resources and even extinction of native species (Oliveira et al., 2015; Fernandes et al., 2016; Jeromini et al., 2018).

The great genetic variability of the species accounts for the low fruit production, therefore few plants flowered and set fruit in the short cycle period. Along the crop cycle, the variation would decrease and yield would increase. It is of note that fruit production in large quantities is not desirable when it is to be used as medicinal plant, since the leaves are the organs used for this purpose (Tirloni et al., 2015; Trevizan et al., 2016).

The high leaf dry mass production and large leaf area of nasturtium were consistent with the growth rate, which was higher in the monocropping with chicken manure. The monocropping contributed to a better use of nutrients provided by the chicken manure. Similar results were found by Sangalli et al. (2004), who observed fresh (5219.69 kg ha⁻¹) and dry (6281.14 kg ha⁻¹) biomass increment of shoots of nasturtium with chicken manure. Moraes et al. (2007) found that the production of monocropped nasturtium was greater in number (13,298,500), fresh mass (8.86 t ha⁻¹), and dry mass (0.70 t ha⁻¹) than intercropped with cabbage cv. “chato de quintal”, indicating a better adaptability in the monocropping.

The production of nasturtium flowers, overall, can be considered low compared with results of other studies...
Vacuum plants. Probably, the greater solubility of aluminum and reducing the vegetative growth capacity and yield of plants diminishes availability of essential nutrients for plants and, consequently, flower production was low. This hypothesis corroborates the results found by Carbonari et al. (2006), who found that nasturtium is a more demanding plant for ZnCl₂ fertilization.

The PCA groups that comprised the vacuum characteristics were created because of the addition of chicken manure, which increased the nutrient content of the soil (Kiehl, 1985), improving N content responsible for photosynthesis, and chlorophyll a and total, thereby improving growth and productivity of plants. The greater foliar P content is involved in energy transfer (ATP), necessary for the photosynthesis, in addition to being the final product of enzymatic reactions of carbohydrate metabolism (Raij, 2017; Taiz et al., 2017).

Without chicken manure, there was a reduction in CEC, diminishing availability of essential nutrients for plants and reducing the vegetative growth capacity and yield of vacuum plants. Probably, the greater solubility of aluminum was toxic and iron increased with acidity, thus, the microorganism population responsible for decomposition of organic matter decreased (Sá et al., 2017).

Therefore, the monocropping of vacuum with chicken manure was more efficient regarding the characteristics assessed, hence, greater growth rate and yield. The PCA groups comprising the nasturtium characteristics occurred because monocropped nasturtium with addition of chicken manure was more responsive to the characteristics assessed.

The intercropping efficiency, regardless of the addition of chicken manure to the soil, evaluated by EAR was greater than 1.0. This resulted from the use of species with different metabolisms and growth cycles. In order to obtain the same production in monoculture it would be necessary increases of 16% and 18% in area. Therefore, the intercropping provides better use of area, nutrients, irrigation, and reduction of cultivation practices.

CONCLUSIONS

Vacuum had greater height growth and production of leaf fresh and dry masses in monocropping with addition of chicken manure to the soil.

Nasturtium had greater growth rate and flower production in monocropping with addition of chicken manure, in the first cultivation cycle.

The intercropping of vacuum and nasturtium was effective, with Equivalent Area Ratios (EAR) of 1.16 and 1.18 without and with chicken manure, respectively.

In this study, the intercropping of vacuum and nasturtium with addition of chicken manure was found feasible.

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